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listing and declared, that it includes no new matter. The applicant has subsequently filed a sequence

<u>54</u> Human G-protein coupled receptor protein cloned form fetal brain CDNA library

a method for screening for compounds which inhibit ligprotein, determination of a ligand to the receptor protein, coding the receptor protein, production of the receptor partial peptide and their salts are disclosed. DNA en-A novel G-protein coupted receptor protein, a

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for such compounds are also disclosed. The receptor screening for candidate compounds of drugs and the protein, its partial peptide and their salts are used for and binding to the receptor protein, a kit for screening



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Description

FIELD OF THE INVENTION

The present invention relates to novel G-protein coupled receptor proteins derived from a human brain, or salts

BACKGROUND OF THE INVENTION

- guanine nucleotide-binding proteins (hereinafter, sometimes, referred to as G-proteins) and are generally called Gin a cell membrane. Many of these receptor proteins mediate signal transmission in a cell by activation of coupled naving seven transmembrane domains. protein coupled receptor proteins or 7-transmembrane receptor proteins because they contain a common structure Many hormones and neurotransmitters regulate functions in a living body through specific receptor proteins existing
- the living body, for example, hormones, neurotransmitters, physiologically active substances and the like. body and play very important roles as targets of molecules which regulate functions of the cells and internal organs o G-protein coupled receptor proteins exist on each functional cell surface of cells and internal organs of a living
- 8 in numerous sites within a brine and regulate the physiological functions thought their corresponding receptor proteins organ such as a brain, its physiological functions are controlled through regulation by many hormones, hormone-like for development of drugs having close relation to such functional mechanisms. For example, in a central nerve system portant means for clarification of functional mechanisms of cells and internal organs of various living body as well as living bodies and their specific receptor proteins, in particular, G-protein coupled receptor proteins provide a very imsubstances, neurotransmitters, physiologically active substances or the like. In particular, neurotransmitters are found To clarify the relation between substances which regulate elaborate functions in cells and internal organs of various
- 25 are subtypes of known receptor proteins. many structures of cDNAs encoding such proteins have not yet been reported. In addition, it is still unknown if there However, it is supposed that many unknown neurotransmitters still exist in a brain and, as for their receptor proteins Also, to clarify the relation between substances which regulate elaborate functions in a brain and their specific
- છ entagonists to receptor proteins, efficiently, in development of drugs, it is required to clarify functional mechanisms of receptor protein genes expressed in a brain and to express them in a suitable expression system. receptor proteins provide a very important means for development of drugs. Further, for screening for agonists and Recently, as a means for analyzing genes expressed in a living body, random analysis of cDNA sequences has
- 딿 functions from their sequential information only. For example, although two ESTs, accession No. T08099 (SEQ ID NO: 5) and No. T27053 (SEQ ID NO: 6), have been registered with the data base, NCBI dbEST, their functions are not Expressed Sequence Tags (ESTs) and are publicly available. However, for many of ESTs, it is difficult to deduce their been studied actively. The sequences of cDNA fragments thus obtained have been registered with data bases as

OBJECTS OF THE INVENTION

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brain, their partial peptides and their salts. One object of the present invention is to provide novel G-protein coupled receptor proteins derived from a human

receptor proteins or their partial peptides. Another object of the present invention is to provide isolated DNA comprising DNA encoding the G-protein coupled

- ŝ having the recombinant vector. A further object of the present invention is to provide a recombinant vector comprising the DNA and a transformant A further object of the present invention is to provide a process for preparing the G-protein coupled receptor proteins
- or their salts Still another object of the present invention is to provide a method for determining ligands to the G-protein coupled
- to the G-protein coupled receptor proteins, a kit for screening for the receptor-agonists or antagonists, the receptorreceptor-agonists or antagonists. agonists or antagonists obtained by the screening and a pharmaceutical composition comprising at least one of the Still another object of the present invention is to provide a method for screening for receptor-agonists or antagonists
- their partial peptides or salts. Yet another object of the present invention is to provide antibodies against the G-protein coupled receptor proteins

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skilled in the art from the following description with reference to the accompanying drawings. These object as well as other objects and advantages of the present invention will become apparent to those

BRIEF EXPLANATION OF DRAWINGS

Fig. 1 is a nucleotide sequence encoding the human G-protein coupled receptor protein (short form) of the present invention obtained in Example 1 hereinalter and its amino acid sequence deduced from the nucleotide sequence.

Fig. 2 is a graph illustrating hydrophobic plotting of the human G-protein coupled receptor protein (short form) of the present invention prepared based on the amino acid sequence of Fig. 1. The parts represented by 1 to 7 are hydrophobic domains.

Fig. 3 is a nucleotide sequence encoding the human G-protein coupled receptor protein (long form) of the present

invention obtained in Example 1 hereinafter and its amino acid sequence deduced from the nucleotide sequence. Fig. 4 is a graph illustrating hydrophobic plotting of the human G-protein coupled receptor protein (long form) of the present invention prepared based on the amino acid sequence of Fig. 1. The parts represented by 1 to 7 are hydrophobic domains.

G-protein coupled receptor protein of the present invention in various human tissues. The value (kb) represents the Fig. 5 illustrates the results of northan hybridization for examining expression levels of mRNA encoding the human size of the RNA molecular weight marker.

SUMMARY OF THE INVENTION

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of G-protein coupled receptor proteins derived from human fetal brain and human adult brain based on two kinds of publicly available EST information registered with a data base whose functions are unknown, and have succeeded in analysis of the entire nucleotide sequences. When their amino acid sequences have been deduced from the nucleotide sequences, the first to the seventh transmembrane domains have been confirmed on hydrophobic plotting. Thus, the As a result of an intensive study, the present inventors have succeeded in isolation of cDNAs encoding two kinds proteins encoded by these cDNAs have been confirmed to be 7-transmembrane type G-protein coupled receptor proteins. The present invention has been completed based on these findings. 20 52

That is, according to the present invention, there are provided:

(1) A G-protein coupled receptor protein which comprises the same or substantially the same amino acid sequence as that represented by SEQ ID NO: 1, or its salt;

(2) The G-protein coupled receptor protein of the above (1) which comprises the same or substantially the same

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amino acid sequence as that represented by SEQ ID NO: 2, or its salt;

(3) A partial peptide of the G-protein coupled receptor protein of the above (1) or its salt;

(4) An isolated DNA comprising DNA having a nucleotide sequence encoding the G-protein coupled receptor

protein of the above (1);

(5) The isolated DNA of the above (4) having the nucleotide sequence represented by SEQ ID NO: 3, (6) The isolated DNA of the above (4) having the nucleotide sequence represented by SEQ ID NO: 4,

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A recombinant vector comprising the DNA of the above (4);

(8) A transformant comprising the recombinant vector of the above (7);

(9) A process for preparing the G-protein coupled receptor protein of the above (1) or its saft which comprises cultivating the transformant of the above (8) to form the G-protein coupled receptor protein; \$

(10) A method for determining a ligand to the G-protein coupled receptor protein of the above (1) or its salt which comprises bringing the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its salt into contact with a test compound;

(11) A method for screening for compounds which after binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises comparing (i) ligand binding upon bringing the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its salt into contact with the ligand, and (ii) that upon bringing the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its salt into contact with the ligand and a test compound;

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(12) A kit for screening for compounds which atter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises as an essential component the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its salt;

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(13) The compounds which after ligand binding to the G-protein coupled receptor protein of the above (1) or its salt obtained by the screening method of the above (11) or the kit of the above (12), or their salts; and

(14) An antibody against the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide

More specifically, the present invention provides:

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I having a deletion of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino 15) The G-prolein coupled receptor protein of the above (1) or its salt, wherein the protein comprises the amino acid sequence represented by SEQ ID NO: 1, a variant of the amino acid sequence represented by SEQ ID NO: acids, a variant of the amino acid sequence represented by SEQ ID NO: 1 having an addition of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids, or a variant of the amino acid sequence represented by SEQ ID NO: 1 having a substitution of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids;

2 having a daletion of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids aguence represented by SEQ ID NO: 2 having an addition of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids, or a variant of the amino acid (16) The G-protein coupled receptor protein of the above (2) or its salt, wherein the protein comprises the amino acid sequence represented by SEQ ID NO: 2, a variant of the amino acid sequence represented by SEQ ID NO: sequence represented by SEQ ID NO: 2 having a substitution of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids;

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(17) The method for determining a ligand of the above (10), wherein the ligand is angiotensin, bombesin, cannabnoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PAC-AP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal and related polypeptides), doparnine, motilin, amylin, bradykinin, CGRP (calcitonin gene related proteins), leukotriene, pancreastacin, prostaglandin, thromboxane, adenosine, adrenalin, α or β-chemokine (e.g., IL-8, GROG, GROB, GROY, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-10, MIP-1|. RANTES, etc.), endothelin, enterogastrin, histarnine, neurotensin, TRH, pancreatic polypeptides or gallamine;

(18) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein or its salt of the above (1), or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount of the labeled ligand bound to the G-protein coupled receptor protein of the above (1) or its salt or the partial peptide of the above (3) or its saft upon bringing the protein of the above (1), the partial peptide of the above (3) or a salt thereof into contact with the labeled ligand, and (ii) that upon bringing the protein of the above (1), the partial peptide of the above (3) or a saft thereof into contact with the labeled ligand and a test compound;

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(19) A method for screening for compounds which atter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount of the labeled ligand bound to cells containing the G-protein coupled receptor protein of the above (1) upon bringing the labeled ligand into contact with the cells with (ii) that upon bringing the labeled ligand and a test compound into contact with the cells;

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(20) A method for screening for compounds which after binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises labeling the ligand, and measuring and comparing (i) an emount of the labeled ligand bound to a membrane fraction of cells containing the G-protein coupled receptor protein of the above (1) upon bringing the labeled ligand into contact with the cell membrane fraction, and (ii) that upon bringing the labeled ligand and a test compound into contact with the cell membrane fraction;

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(21) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount of the labeled ligand bound to the G-protein coupled receptor protein expressed on the cell membrane of the transformant of the above (8) by cultivating the transformant upon bringing the labeled ligand into contact with the expressed G-protein coupled receptor protein, and (ii) that upon bringing the labeled ligand and a test compound into contact with the expressed G-protein coupled receptor protein;

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(22) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises measuring and comparing (i) a cell stimulation activity mediated by the G-protein coupled receptor protein upon bringing a compound which activates the G-protein coupled receptor protein of the above (1) or its salt into contact with cells containing the G-protein coupled receptor protein of the above (1), and (ii) that upon bringing the compound which activates the G-protein coupled receptor protein or its salt and a test compound into contact with the cells;

(23) A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of the above (1) or its salt, or their salts which comprises measuring and comparing (i) a cell stimulation activity mediated by the G-protein coupled receptor protein upon bringing a compound which activates the G-protein coupled receptor protein of the above (1) or its salt into contact with the G-protein coupled receptor protein expressed on the cell membrane of the transformant of the above (8) by cultivating the transformant, and (ii) that upon bringing the compound which activates the G-protein coupled receptor protein or its salt and a test compound into contact with the G-protein coupled receptor protein expressed on the cell membrane;

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(24) The method for screening of the above (22) or (23), wherein the compound which activates the G-protein coupled receptor protein of the above (1) is angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, se-

prostaglandin, thromboxane, adenosine, adrenalin, α or β-chemokine (e.g., IL-8, GROα, GROβ, GROγ, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.), endothelin, enteroides), dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related proteins), leukotriene, pancreastacin adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vascactive intestinal and related polypeprotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, gastrin, histamine, neurotensin, TRH, pancreatic polypeptides or gallamine

(25) Compounds which alter binding of a ligand to the G-prolein coupled receptor protein of the above (1) or its sall obtained in any one of the method of screening of the above (11) and (18) or (24), or their salls;

(27) The screening kit of the above (12) comprising cells containing the G-protein coupled receptor protein of the (26) A pharmaceutical composition comprising the compound of the above (25) or its salt;

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(28) The screening kit of the above (12) comprising a membrane fraction of cells containing the G-protein coupled receptor protein of the above (1);

obtained by using the screening kit of the above (12), (27) or (28), or its salt; (29) The compound which alter ligand binding to the G-protein coupled receptor protein of the above (1) or its salt

G-protein coupled receptor protein of the above (1), the partial peptide of the above (3) or a salt thereof. peptide of the above (3) or its salt which comprises bringing the antibody of the above (14) into contact with the (31) A method for determining the G-protein coupled receptor protein of the above (1), or its salt or the partial (30) A pharmaceutical composition comprising the compound of the above (29) or its salt; and

DETAILED DESCRIPTION OF THE INVENTION

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by SEQ ID NO: 1 (Fig. 1) and it may be the receptor protein having the same or substantially the same amino acid sequence as that represented by SEQ ID NO: 2 (Fig. 3). The amino acid sequence of SEQ ID NO: 2 is a variant of the protein") is the receptor protein which has the same or substantially the same amino acid sequence as that represented amino acid sequence represented by SEQ ID NO: 1 having an addition of 61 amino acids at the N-terminal end of SEQ The G-protein coupled receptor protein of the present invention (hereinafter sometimes abbreviated to *receptor

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မ္ပ છ å another mammal (e.g., guinea pig, rat, mouse, chicken, rabbit, pig, sheep, callle, monkey, etc.), for example, splenic cancer cells thereof and the like; and any tissues containing such cells, for example, brain, various parts of brain (e g., olfactory bulb, amygdala, cerebral basal ganglia, hippocampus, thalamus, hypothalamus, substhanlamic nucleus osteocyte, osteoblast, osteoclast, mammary gland cell, hepatocyte, or interstitial cells or precursor cells, stem cells or cell, mast cell, neutrophil, basophil, eosinophilic leukocyte, monocyte, etc.), megakaryocyte, synovial cell, chondrocyte endothelial cell, fibroblast, fibrocyte, muscular cell, fat cell, immunocyte (e.g., macrophage, T cell, B cell, natural killer cell, nerve cell, glia cell, β cell of pancreas, marrow cell, mesangial cell, Langerhans' cell, epidermic cell, epithelial cell, ÖNÖ. peripheral blood, peripheral blood leukocyte, intestinal tract, prostate, testicle, testis, ovarium, placenta, uterus, bone marrow, adrenal gland, skin, muscle, lung, digestive tract, blood vessel, heart, thymus, spleen, submandibular gland, stantia nigra), spinal cord, pituitary, stomach, pancreas, kidney, liver, genital organs, thyroid gland, gallbladder, bone cerebral cortex, medulta, cerebellum, occipital pole, frontal lobe, pulamen, caudate nucleus, corpus callosum, subint, small intestine, large intestine, skeletal muscle and the like, in particular, brain and various parts of brain. And The receptor protein of the present invention may be eny peptide derived from any cells of a human being and

ቴ or SEQ ID NO: 2 and having substantially the same activity as that of the receptor protein comprising the amino acid homology, more preferably at least about 90% homology to the amino acid sequence represented by SEQ ID NO: 1 or SEQ ID NO. 2" includes any protein which has at least about 70% homology, preferably at least about 80% peptide may be a synthetic one. The wording "the same or substantially the same as the amino acid sequence as that represented by SEQ ID NO

sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2. Therefore, quantitative factors such as degrees of ligand binding activity and signal information transmission activity and the like. The wording "substantially the same" means that the natures of their activities are equal to one another Examples of substantially the same activity include ligand binding activity, signal information transmission activity

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an addition of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids, or a sequence represented by SEQ ID NO: 2 having a deletion of one or more, preferably about 1 to about 30, more preferably about 1 to about 10 amino acids, a variant of the amino acid sequence represented by SEQ ID NO: 2 having may differ from one another. about 1 to about 30, more preferably about 1 to about 10 amino acids. variant of the amino acid sequence represented by SEQ ID NO: 2 having a substitution of one or more, preferably Further, the receptor protein of the present invention may be a protein comprising a variant of the amino acid

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More specifically, the receptor protein of the present invention includes, for example, the receptor protein compris

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ing the amino acid sequence represented by SEQ ID: NO 1 derived from a human brain, or the G-protein coupled receptor protein comprising the arnino acid sequence represented by SEQ ID: NO 2 derived from a human brain.

ö of an amino acid in the molecule of the above receptor protein is protected with a suitable protecting group (e.g., acy) a substituent (e.g., -OH, -COOH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chain region of the above receptor protein is cleaved in a living body and the glutarnyl group formed is pyroglutaminated; or a protecting group (e.g., acyl group having 1 to 6 carbon atoms such as formyl group, acetyl group, etc.); the N4erminal protein, wherein the amino group of the N-terminal methionine residue of the above receptor protein is protected with group having 1 to 6 carbon atoms such as formyl group, acelyl group, etc.), or conjugated proteins of the above receptor protein such as glycoproteins having sugar chains Furthermore, examples of the receptor protein of the present invention include variants of the above receptor

left hand end (amino terminal) is the N-terminal and the right hand end (carboxyl terminal) is the C-terminal. And, in 6 to 12 carbon atoms such as phenyl, α -naphthyl, etc., an aralkyl having 7 to 14 carbon atoms such as a phenyl- $C_{1,2}$ butyl, etc., a cycloalkyl group having 3 to 8 carbon atoms such as cyclopentyl, cyclohexyl, etc., an aryl group having R of the ester group include an alkyl group having 1 to 6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-(-COOH) or carboxylate (-COO-), but the C-terminal may be the arnide (-CONH₂) or an ester (-COOH). Examples of the amino acid sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2, normally, the C-terminal is carboxyl group the receptor protein of the present invention, a representative example thereof being the receptor protein comprising The receptor protein of the present invention is represented by a conventional manner in peptide art. That is, the

20 the C-terminal, it may be amidated or esterified and such amide or ester is also included in the scope of the receptor addition, pivaloyloxymethyl ester or the like which is used widely as an ester for oral administration can also be used. alkyl group (e.g., benzyl, phenethyl, etc.), an α -naphthyl- $G_{1,2}$ alkyl group (e.g., α -naphthylmethyl, etc.) and the like. In protein of the present invention. The ester group may be the same group as that described with respect to the above When the receptor protein of the present invention has a carboxyl group (or carboxylate) at a position other than

sell is preferred. Examples of the salt include those with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrochloric acid, etc.) and those with organic acids (e.g., acetic acid, formic acid, propionic acid, furnaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesultonic acid, benznesuttonic acid, etc.). As the salt of the receptor protein of the present invention, in particular, a physiologically acceptable acid addition

છ of a human being or another mammal by a per se known purification method of proteins. Alternatively, the receptor receptor protein of the present invention or according to a peptide synthesis method as described hereinafter. protein or its salt of the present invention can be prepared by cultivating a transformant containing DNA encoding the The receptor protein or its salt of the present invention can be prepared from the above described cells and tissues

ક્ષ and then extracted with, for example, an acid. The extract can be purified and isolated by combining chromatographies such as reverse phase chromatography, ion exchange chromatography and the like. When it is produced from cells or tissues of a human being or another mammal, the cells or tissues are homogenized

8 invention, for example, a part of the receptor protein molecule of the present invention which is exposed to outside of 4. A peptide containing a hydrophobic domain part can be similarly used. In addition, the peptide may contain each been analyzed to be extracellular domains (hydrophilic domains) in the hydrophobic plotting analysis as shown by Flg receptor protein having the amino acid sequence represented by SEQ ID NO: 2 is that containing the parts which have domains (hydrophilic domains) in the hydrophobic plotting analysis as shown by Fig. 2. The partial peptide of the sequence represented by SEQ ID NO: 1 is a peptide containing the parts which have been analyzed to be extracellular a cell membrane or the like can be used. Specifically, the partial peptide of the receptor protein having the amino acid As the partial peptide of the receptor protein (hereinafter sometimes abbreviated to "partial peptide") of the present

છ sequence represented by SEQ ID NO: 2. amino acids, the 254th to 265th amino acids, the 335th to 368th and the 448th to 459th amino acids of the amino acid the 193rd to 204th amino acids, the 274th to the 307th amino acids, and the 387th to 398th amino acids of the amino domain separately or plural domains together. acid sequence represented by SEQ ID NO: 1 as well as those having the amino acid eequences of the 139th to 191st Examples of the partial peptide include those having the amino acid sequences of the 78th to 130th amino acids

a substituent (e.g., -OH, -COOH, amino group, imidazole group, indole group, guanidino group, etc.) on the side chain peptide, wherein the amino group of N-terminal methionine residue of the above receptor protein is protected with a protecting group (e.g., acyl group having 1 to 6 carbon atoms such as formyl group, acetyl group, etc.); the N-terminal region of the above receptor protein is cleaved in a living body and the glutarnyl group formed is pyroglutaminated; or Further, the partial peptide of the receptor protein of the present invention include variants of the above partial

of an amino acid in the molecule of the above receptor protein is protected with a suitable protecting group (e.g., acyl group having 1 to 6 carbon atoms such as formyl group, acetyl group, etc.), or conjugated peptides of the above partial peptide such as glycopeptides having sugar chains.

Normally, the C-terminal of the partial peptide of the present invention is a carboxyl group (-COCH) or carboxylate (-COCH) and, like the receptor protein of the present invention, the C-terminal may be the amide or ester. When the partial peptide of the present invention has a carboxyl group (or carboxylate) at a position other than the C-terminal, it may be amidated or esterified and such amide or ester is also include in the scope of the partial peptide of the present invention. The ester group may be the same group as that described with respect to the above C-terminal of the receptor models.

As the salt of the partial peptide of the present invention, in particular, a physiologically acceptable acid addition salt is proferred. Examples of the salt include those with inorganic acids (e.g., hydrochloric acid, culture acid, etc.) and those with organic acids (e.g., acetic acid, sumic acid, surface acid, formic acid, formic acid, furnaric acid, acid

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The partial peptide or its salt of the present invention can be prepared according to a per se known peptide synthesis method or by cleaving the receptor protein of the present invention with a suitable peptidase.

As the peptide synthesis method, for example, any of solid phase synthesis and liquid phase synthesis can be employed. That is, the objective peptide can be produced by condensing a partial peptide or amino acid sequence which can compose of the partial peptide of the produced by condensing a maning part and deprotecting a protecting group, if any. Conventional condensing aminods and deprotecting methods can be employed and they are described by, for example, M. Bodanszky and M.A. Ondetti, Peptide Synthesis, inlenscience Publishers, New York (1965). Schnoeder and Luebke, The Peptide, Academic Press, New York (1965); Nobuo Izumi et al., Fundamental and Experiment of Peptide Synthesis, Maruzen (1975); Haruaki Yazima and Syunpei Skakibara, Biochemistry Experiment Lecturion, Protein Chemistry IV, 205 (1977); Haruaki Yazima, Second Series Drug Development Vol. 14, Peptide Synthesis, Hirokawa Shoton.

After completion of the reaction, the partial paptide of the present invention can be purified and isolated by combining conventional purification methods such as solvent extraction, distillation, column chromatography, liquid chromatography, recrystalifization and the like. In case the partial peptide thus obtained is a free peptide, it can be converted into its appropriate salt according a known method. On the other hand, the peptide obtained is in the form of a salt, it can be converted into the corresponding these peptide.

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The DNA encoding the receptor protein of the present invention may be any DNA in so far as it contains the nucleotide sequence encoding the above-described receptor protein of the present invention. The DNA may be any of genomic DNA, genomic DNA theray, cDNA derived from the above-described cells and tissues, cDNA library derived from the above-described cells and tissues, cDNA library derived from the above-described cells and tissues and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid, phagemid and the tike, in addition, the DNA can be amplified by reverse transcriptase polymerase chain reaction (hereinatiler abbreviated to RT-PCR) with a mRNA fraction prepared from the above-described cells and tissues.

Specifically, the DNA encoding the receptor protein having the same or substantially the same amino acid sequence represented by SEO ID NOT: to the present invention may be, for example. DNA having the nucleotide sequence represented by SEO ID NOT: or any DNA having a nucleotide sequence hybridizable to the nucleotide sequence represented by SEO ID NOT: a under high stringent conditions and encoding a receptor protein which has the same activities, i.e., ligand binding activity, signal information transmission activity and the like as those of the receptor protein peptide having the armino acid esquence represented by SEO ID NOT: Examples of the hybridizable DNA include DNA having at least about 10% to about 80% homology, preferably, at least about 90% homology to the nucleotide sequence represented by SEO ID NOT: 3.

More specifically, as the DNA encoding the receptor protein containing the amino acid sequence represented by SEQ ID NO: 1, the DNA having the nucleotide sequence represented by SEQ ID NO: 3 or the like can be used.

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The DNA encoding the receptor protein having the same or substantially the same arritino acid sequence represented by SEQID NO: 2 of the present invention may be, for example, DNA having the nucleotide sequence represented by SEQID NO: 4 or any DNA having a nucleotide sequence hybridizable to the nucleotide sequence represented by SEQID NO: 4 under high stringent conditions and encoding a receptor protein which has the same activities, i.e., ligand binding activity, signal information transmission activity and the like as those of the receptor protein peptide having the arrino acid sequence represented by SEQID NO: 2. Examples of the hybridizable DNA include DNA having at least about 70% to about 80% homology, preferably, at least about 90% nonology, more preferably, at least about 95% homology to the nucleotide sequence represented by SEQID NO: 4.

More specifically, as the DNA encoding the receptor protein containing the amino acid sequence represented by SEQ ID NO: 2, the DNA having the nucleotide sequence represented by SEQ ID NO: 4 or the like can be used. The nucleotide sequence represented by SEQ ID NO: 4 is a variant of the nucleotide sequence of SEQ ID NO: 3 having an addition of 183 bases at its 5'-lerminal.

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Hybridization can be carried out by a per se known method or its modification, for example, under high stringent notitions.

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The high stringent conditions used herein are, for example, those of socium concentration at about 19 mM to about 40 mM, preferably about 19 mM to about 20 mM and a temperature at about 50°C to about 10°C, preferably about 60°C to about 65°C, in particular, pybridization conditions of sodium concentration at about 19 mM and a temperature at about 65°C are most preferred.

The DNA encoding the partial peptide of the present invention may be any DNA in so far as it contains the nucleotide sequence encoding the above-described partial peptide of the present invention. The DNA may be any of genomic DNA, genomic DNA tibray, collarA derived from the above-described cells and tissues, cDNA library derived from the above-described cells and tissues and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid, phagemid and the like, In addition, the DNA can be amplified by reverse transcriptase polymerase shahin reaction the above-described cells and shared and the like. In addition, the DNA can be amplified by reverse transcriptase polymerase abhin reaction (hereinalter abbreviated to FT-PCR) with a mRNA fraction prepared from the above-described cells and desirance.

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Specifically, the DNA encoding the partial peptide of the receptor protein having the same or substantially the same amino acid sequence representant by SEQ ID NO; 1 of the present invention may be, for example, DNA having the nucleotide sequence represented by SEQ ID NO; 3 or any DNA having a nucleotide sequence hybridizable to the nucleotide sequence represented by SEQ ID NO; 3 under high stringent conditions and encoding a receptor protein which has the same activities, i.e., ligand binding activity, signal information transmission activity and the like as those of the receptor protein having the amino acid sequence represented by SEQ ID NO; 1. Examples of the hybridizable DNA include DNA having at least about 170% to about 80% homology, preferably, at least about 95% homology to the nucleotide sequence represented by SEQ ID NO; 2. Examples of the hybridizable DNA include DNA having at least about 95% homology to the nucleotide sequence represented by SEQ ID NO; 3.

For example, as the DNA encoding the partial poptide of the receptor protein containing the amino acid sequence represented by SEQ ID NO: 1, the DNA having the nucleotide sequence represented by SEQ ID NO: 3 or the like can be used. More specifically, as the DNA encoding the partial poptide having the amino acid sequences of the 78th to 130th amino acids, the 193rd to 204th amino acids, the 274th to the 307th amino acids so the 387th to 388th amino acids of the amino acid sequence represented by SEQ ID NO: 1, the DNA having the nucleotide sequence of the 232nd to 390th bases, the 577th to 612th bases, the 820th to 921st bases or the 1159th to 1194th bases of the nucleotide sequence of the sequence represented by SEQ ID NO: 3 can be used.

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The DNA encoding the partial peptide of the receptor protein having the same or substantially the same amino acid sequence represented by SECI DNO: 2 of the present invention may be, for example, DNA having the nucleotide sequence represented by SECI DNO: 4 or any DNA having a nucleotide sequence hybridizable to the nucleotide sequence represented by SECI DNO: 4 under high stingent conditions and encoding a receptor protein which has the same activities, i.e., ligand binding activity, signal information transmission activity and the like as those of the receptor protein peptide having the armino acid sequence represented by SECI DNO: 2. Examples of the hybridizable DNA include DNA having at least about 70% to about 90% homology, preferably, at least about 90% homology to the nucleotide sequence represented by SECI DNO: 2. Examples of the hybridizable preferably, at least about 95% homology to the nucleotide sequence represented by SECI DNO: 4.

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For example, as the DNA encoding the partial peptide of the receptor protein containing the amino acid sequence represented by SEQ ID NO: 2, the DNA having the nucleotide sequence represented by SEQ ID NO: 4 or the like can be used. More specifically, as the DNA encoding the partial peptide having the amino acid sequences of the 139th to 191th amino acids, the 254th to 25th amino acids, the 335th to the 368th amino acids or the 448th to 459th amino acids or the 448th to 459th amino acids or the article of the amino acid sequence represented by SEQ ID NO: 2, the DNA having the nucleotide sequence of the 415th to 573th bases, the 760th to 795th bases, the 1003rd to 1104th bases or the 1342nd to 1377th bases of the nucleotide sequence represented by SEQ ID NO: 4 can be used.

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Hybridization can be carried out by a per se known method or its modification, for example, under high stringent conditions.

As described above, the high stringent conditions used herein are, for example, those of sodium concentration at about 19 mM to about 40 mM, preferably about 19 mM to about 20 mM, and a temperature at about 50°C to about 60°C to nearly about 60°C to nearly about 60°C to about 60°C. In particular, hybridization conditions of sodium concentration at about 19 mM and a temperature at about 65°C are most preferred.

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As the means for cloning the DNA encoding the entire receptor protein of the present invention, there is amplification by PCR using synthetic DNA primers containing partial nucleotide sequences of the receptor protein of the present invention. Alternatively, DNA integrated into a suitable vector is selected by hybridization with labeled DNA tragment or a synthatic DNA encoding a part or entire region of the receptor protein of the present invention. The hybridization is carried out, for example, according to the method described in Molecular Cloning 2nd Ed., J. Samrook et al., Cold Spring that be tress, I press, (1989). A commercially available library can be used according to the direction of the attached manufacuer's protocol.

Conversion of the nucleotide sequence of DNA can be carried out according to a per se known method such as Gupped douplex method or Kunkel method or its modification by using a known kit, Mutan 14-K, or Mutan 14-K, (both Takara Shuzo Co., Ltd., TM represents trademark). The nucleotide sequence represented by SEO ID NO: 1 can be produced by deleting 183 based from the 5'-terminal of the nucleotide sequence represented by SEO ID NO: 2.

by using a suitable synthetic DNA adapter. have the codon, ATG, as a translation initiation codon at its 5' terminal side and the codon, TAA, TGA or TAG if desired, it can be used after digestion with one or more restriction enzymes or a linker can be added. The DNA may translation termination codon at its 3' terminal side. These translation initiation and termination codons can be added The cloned DNA encoding the receptor protein can be used as such according to a particular purpose. Alternatively,

DNA fragment to a suitable expression vector at the downstream from a promoter in the vector. out the desired DNA fragment from the DNA encoding the receptor protein of the present invention and (b) joining the The expression vector of the receptor protein of the present invention can be prepared, for example, by (a) cutting

derived from Bacillus subtilis (e.g., pUB110, pTP5, pC194), plasmids derived from yeast (e.g., pSH19, pSH15), bacpXT1, pRc/CMV, pRc/RSV, pcDNAI/Neo, etc. teriophages such as λ phage, etc., animal viruses such as retrovirus, vaccinia virus, baculovirus, etc. as well as pA1-11, Examples of the vector include plasmids derived form E. coli (e.g., pBR322, pBR325, pUC12, pUC13), plasmids

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gene expression. In case of using animal cells as the host, examples of the promoter include SRlpha promoter, SV40 promoter, etc. In case of using insect cells as the host, preferred examples of the promoter include polyhedrin prompter yeast as the host, preferred examples of the promoter include PHO5 promoter, PGK promoter, GAP promoter, ADH Bacilius as the host, preferably, SPO1 promoter, SPO2 promoter, penP promoter, etc. can be used. In case of using fp promoter, lac promoter, recA promoter, λ P_L promoter, lpp promober, etc. In case of using bacteria of the genus romoter, LTR promoter, CMV promoter, HSV-TK promoter, etc. Among them, CMV promoter or SRa promoter is eferred. In case of using bacteria of the genus <u>Escherichia</u> as the host, preferred examples of the promoter include The promoter used in the present invention may be any promoter in so far as it matches with a host to be used to

of the selection marker include dihydrofolate reductase (hereinafter sometimes abbreviated to dhir) gene [methotrexate with DHFR gene as the selection marker, selection can also be carried out by using a thymidine free medium. (hereinafter sometimes abbreviated to Neo, G418 resistance), etc. In particular, when CHO (dhir) cell is used together (MTX) resistance], ampicillin resistant gene (hereinafter sometimes abbreviated to Amp1), neomycin resistant gene addition signal, selection marker, SV40 replication origin (hereinalter sometimes abbreviated to SV40 ori) etc. Examples In addition to the above, optionally, the expression vector may further contains enhancer, splicing signal, poly A

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signal sequence, antibody motecule signal sequence, etc. in case of using animal cells as the host, respectively. subtitisin signal sequence, etc. in case of using bacteria of the genus Bacillus as the host; mating factor α signal OmpA signal sequence, etc. in case of using bacteria of the genus Escherichia as the host; α-amylase signal sequence of the present invention. As the signal sequence, there may be mentioned alkaline phosphatase signal sequence sequence, invertase signal sequence, etc. in case of using yeast as the host; insulin signal sequence, α-interferon If necessary, a signal sequence which matches with a host is added to the N-terminal side of the receptor protein

The DNA encoding receptor protein of the present invention thus constructed can be employed to transform the

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yeast, insect cells, insects and animal cells, etc. Specific examples of bacteria of the genus Escherichia include Escherichia coli K12 DH1 [Proc. Natl. Acad. Sci As the host, for example, there may be mentioned bacteria of the genus Escherichia, bacterial of the genus Bacillus

(1978)], HB101 [Journal of Molecular Biology, 41, 459 (1969)], C600 [Genetics, 39, 440 (1954)], etc. Examples of bacteria of the genus Bacillus include Bacillus subtilis MI 114 (Gene, 24, 255 (1983)), 207-21 (Journal

USA, <u>60</u>, 160 (1968)], JM103 [Nucleic Acids Research, <u>9</u>, 309 (1981)], JA221 [Journal of Molecular Biology, <u>120</u>, 517

Biochemistry, 95, 87 (1984)], etc.

ŝ the SI cell, for example, SI9 cell (ATCC CRL1711) and SI21 cell described by Vaughn, J. L., in Vitro, 13, 213-217 (1977) Examples of insect cells include <u>Spodoplera frugiperda</u> cell (SI cell), MG1 cell derived from mid-intestine of <u>Tri-choplusia ni</u>, High Five ^{nu} cell derived from egg of <u>Trichoplusia ni</u>, cells derived from <u>Mamestra brassicae</u>, cells derived from Estigmena acrea, etc. for the virus, AcNPV; and Bombyx mori N cell (BmN cell), etc. for the virus, BmNPV. As Examples of yeast include Saccaromyces cereviseae AH22, AH22, NA87-11A, DKD-5D, 20B-12, etc.

can be used.

50 Chinese hamster cell CHO (dhfr CHO cell), mouse L cell, mouse AtT-20, mouse myeloma cell, rat GH 3, human FL Examples of animal cells include monkey cell COS-7, Vero, Chinese hamster cell CHO, DHFR gene deficient As the insect, for example, a larva of Bombyx mori can be used [Maeda et al., Nature, 315, 592 (1985)]

in Proc. Natl. Acad. Sci. USA, 69, 2110 (1972) or Gene, 17, 107 (1982) Transformation of bacteria of the genus Escherichia is carried out, for example, according to the method described

જ Molecular & General Genetics, 168, 111 (1979). Transformation of bacteria of the genus Bacillus is carried out, for example, according to the method described in

Transformation of yeast is carried out, for example, the method described in Proc. Natl. Acad. Sci. USA, 75, 1929

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Technology, 6, 47-55(1988). Transformation of insect cells or insect is carried out, for example, according to the method described in Biol

Transformation of animal cells is carried out, for example, according to the method described in Virology, 52, 456

Thus, the transformant transformed with the expression vector containing the DNA encoding the G-protein coupled

ŏ liquid culture medium and materials required for growth of the transformant such as carbon sources, nitrogen sources salts, nitrate salts, com steep liquor, peptone, casein, meat extract, soybean meat, potato extract, etc. The inorganic starch, sucrose, etc. The nitrogen sources include, for example, inorganic or organic materials such as ammonium inorganic materials, etc. are added to the medium. Examples of the carbon sources include glucose, dextrin, soluble In case of the bacterial host of the genus Escherichia or Bacillus, the transformant can be suitably cultivated in a

3 glucose and Casamino Acids (Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor yeast, vitamins, growth promoting lactors etc. can be added. Preferably, the medium is adjusted to pH about 5 to about 8. materials include, for example, calcium chloride, sodium dihydrogen phosphate, magnesium chloride, etc. In addition aboratory. New York, 1972). If necessary, in order to activate the promoter efficiently, for example, an agent such as Preferably, the medium for cultivating the bacteria of the genus Escherichia is, for example, M9 medium containing

about 43°C for about 3 hours to about 24 hours. If necessary, the culture can be aerated or stirred. In case of the bacterial host of the genus Escherichia, normally, the transformant is cultivated at about 15°C to

3β-indotyl acrylic acid can be added to the medium.

40°C for about 6 hours to about 24 hours. If necessary, the culture can be aerated or stirred In case of the bacterial host of the genus Bacilius, normally, the transformant is cultivated at about 30°C to about

25 the transformant is cultivated at about 20°C to about 35°C for about 24 hours to about 72 hours. If necessary, the L. et al., Proc. Natl. Acad. Sci. USA, 77, 4505 (1980) and SD medium containing 0.5% Casamino Acids [Bitter, G. A., Proc. Natt. Acad. Sci. USA, 81, 5330 (1984)]. Preferably, the medium is adjusted to pH about 5 to about 8. Normally, In case of the yeast host, the transformat is cultivated in, for example, Burkholder's minimal medium [Bostian, K

culture can be aerated or stirred.

မ about 27°C for about 3 days to about 5 days and, if necessary, the culture can be aerated or stirred [Grace, T. C. C.,. Nature, <u>195</u>, 788 (1962)] to which an appropriate additive such as inactivated 10% bovine serum is added. Preferably, the medium is adjusted to pH about 6.2 to about 6.4. Normally, the transformant is cultivated at In case of the insect cell host or insect host, the transformat is cultivated in, for example, Gace's Insect Medium

culture can be aerated or stirred the transformant is cultivated at about 30°C to about 40°C for about 15 hours to about 60 hours and, if necessary, the for the Biological Medicine, 73, 1 (1950)], etc. Preferably, the medium is adjusted to pH about 6 to about 8. Normally, to about 20% fetal bovine serum [Science, 122, 501 (1952)], DMEM medium [Virology, 8, 396 81959)], RPMI 1640 medium [The Journal of the American Medical Association, 199, 519 (1967)], 199 medium [Proceeding of the Society In case of the animal cell host, the transformant is cultivated in, for example, MEM medium containing about 5%

cell membrane of the transformant. As described hereinabove, the G-protein coupled receptor protein of the present invention can be produced at the

6 out, for example, as follows Separation and purification of the receptor protein of the present invention from the above culture can be carried

or a surfactant such as Trilon X-100", etc. When the receptor protein is secreted in the culture broth, after completion protein by centrifugation, filtration, etc. The buffer may contain a protein modifier such as urea, guanine hydrochloride cation, treatment with lysozyme and/or freeze-thaw cycling, followed by separating a crude extract of the receptor and suspended in a suitable buffer. Then, the transformant is disrupted by a per se known method such as ultrasoniappropriate known method. For example, after cultivation, the transformant is recovered by a per se known method Extraction of the receptor protein of the present invention from the transformant culture can be carried out by an

of cultivation, its supernatant can be separated from the transformant cells to collect the supernatant

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filtration, SDS-polyacrylamide gel electrophoresis, etc.; a method utilizing difference in electric charges such as ion and purification methods, there may be mentioned a method utilizing difference in solubilities such as salting out, out by combining per se known separation and purification methods appropriately. As the per se known separation solvent precipitation, etc.; a method mainly utilizing difference in molecular weights such as dialysis, uttrafiltration, gei Purification of the receptor protein contained in the culture supernatant thus obtained or the extract can be carried

g g etc.; a method utilizing difference in isoelectric points such as isoelectric point electrophoresis; and the like, a method utilizing difference in hydrophobic properties such as reverse phase high performance liquid chromatography, exchange chromatography, etc.; a method utilizing difference in specific affinities such as affinity chromatography, etc.;

ification. On the other hand, when the receptor protein is obtained in the form of a salt, it can be converted into the free receptor protein or a different salt by a per se known method or its modification When the free receptor protein is obtained, it can be converted into its salt by a per se known method or its mod-

The receptor protein produced by the recombinant can be treated with an appropriate protein modifying enzyme prior to or after purification to appropriately modify the protein or to partially remove a polypeptide. Examples of the protein modifying enzyme include typsin, chymotrypsin, ariginyl endopeptidase, protein kinase, glycosidase and the

The activity of thus-produced receptor protein or its salt of the present invention can be determined by a binding test with a labeled ligand or an enzyme immunoassay with a specific antibody.

The receptor protein, its partial peptide or their salts, and DNAs encoding them of the present invention can be employed (a) in a method for determination of a ligand to the receptor protein of the present invention, (b) for obtaining an antibody or an antiserum, (c) for construction of a expression system of the recombinant receptor protein, (d) for development of an receptor protein, especially an expression system of the recombinant leveloper protein, (d) for development of an receptor protein government of the receptors sion system, (e) for practice of drug design based on comparison with structurally analogous ligands and receptors, (f) as reagents for preparation of probes to be used in gene diagnosis, PCR primers, etc., (g) as drugs for gene prophylaxis and then like.

In particular, screening for agonists or antagonists to the G-protein coupled receptor protein which are specific to a human being and another mammal can be carried out by using a receptor binding assay system utilizing an expression system of the recombinant G-protein coupled receptor protein of the present invention, and the agonists and antagonists can be used as prophylactic and thorapeutic drugs for various diseases.

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More specific description of the use of the receptor protein, its partial peptide or their salts, DNAs encoding the receptor protein or its partial peptide and antibody will be set forth below.

(i) Determination method of a ligand to the G-protein receptor protein

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The receptor protein of the present invention or its sail, or the partial protein or its sail of the present invention is useful as a reagant to investigation or determination of alligand to the receptor protein or its sail of the present invention. That is, the present invention provides a melhod of determination of a ligand to the receptor protein of the present invention containing bringing the resopotor protein or its partial peptide of the present invention or a sail thereof into

contact with a test compound.

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Examples of the test compound include tissue extract, cell culture supernalant of a human being or another manmat (e.g., mouse, rat, pig, cattle, sheep, monkey, etc.), or the like, in addition to the above-described known ligands, such as angiotensin, bombesin, cameabraid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide V, optiol, purine, vasopressin, oxytocin, PACAP, serotein, glutamine, astrotonin, melatonin, neuropeptide V, optiol, purine, vasopressin, oxytocin, PACAP, serotein, glutagon, calcitonin, adrenomedullin, sornatostatin, clAPH, CPF, ACTH, GRP, PTH, MP (vasoactive intestinal and related polypeptides), dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related proteins), leukotriene, pancreastatoin, prostaglandin, thomboxane, adenosisin, adrenalin, or glahemnokine (e.g., IL-8, GROc, GROß, GROY, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1c, mIP-1β, RAMTES, etc.), endothelin, anterogastinin, histemine, neurotensin, TRH, pancreastic polypeptides or gallamine. For example, the issue astractic or the cell culture supematant is added to the receptor protein of the present invention and the mixture is fractionated by measuring a cell stimulation activity, etc. to finally obtain a single ligand.

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Specifically, the ligand determination method of the present invention is carried out by using the receptor protein or its partial peptide of the present invention or a sell thereof, or by constructing an expression system of the recombinant receptor protein and using a receptor binding assay system utilizing the expression system to determine a compound (e.g., peptide, protein, non-peptide compound, synthetic compound, lementation product, etc.) showing a cell stimulation activity (e.g., an activity to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of intracellular CaP4, formation of intracellular cAMP; formation of intracellular cAMP; production of inositol phosphate, change of cell membrane potential; Nospholytation of intracellular protein, activation of c-fos, lowering of pH, etc.) upon binding to the receptor protein of the present invention.

The ligand determination method of the present invention is characterized by measurement of, for example, an amount of a test compound bound to the receptor protein or the partial peptide or a cell stimulation activity upon bringing the receptor protein or the partial peptide into contact with the test compound.

o receptor protein or the partial peptide into contact with the test compound: More specifically, the ligand determination method of the present invention is:

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(a) a method for determination of a ligand to the receptor protein or its ealt of the present invention which comprises labeling a test compound, and measuring an amount of the labeled test compound bound to the receptor protein, the partial peptide or their salts upon bringing the receptor protein, the partial peptide or their salts into contact with the beheled test compound;

(b) a method for determination of a ligand to the receptor protein or its salt of the present invention which comprises labeling a test compound, and measuring an amount of the tabeled test compound bound to cells containing the receptor protein or a membrane fraction of the cells upon bringing the labeled test compound into contact with the cell or the cell membrane fraction;

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(c) a method for determination of a ligand to the receptor protein which comprises labelling a test compound, and measuring an amount of the labeled test compound bound to the G-protein coupled receptor protein expressed on the cell membrane of a transformant containing the DNA encoding the receptor protein of the present invention by cultivating the transformant upon bringing the labeled test compound into contact with the expressed G-protein coupled receptor profesi.

of a methodro determination of a ligand to the receptor protein or its salt of the present invention which comprises measuring a cell strendation activity (e.g., an activity to enhance or inhibit release of arachidonic acid, release of acety choine, release of intracellular Ca²+, formation of intracellular cAAMP, formation of intracellular callular protein, activation duction of inositol phosphate, change of cell membrane potential, phospholylation of intracellular protein, activation of c-los, lowering of pH, etc.) mediated by the receptor protein upon bringing a test compound into contact with

cells containing the receptor protein; (e) a machine to the receptor protein or its saft which comprises which comprises (e) a method for determination of a ligand to the receptor protein or inhibit release of arachidonic acid, release of measuring a cell stimulation acidity (e.g., an activity to enhance or inhibit release of arachidonic acid, release of acety choline, release of intracellular Ca²⁺, formation of intracellular cAMP; formation of intracellular cBMP, production of inositol phosphate, change of cell membrane potential, phospholylation of intracellular protein, activation of close, towning of pt., lact), mediated by the receptor protein upon bringing a test compound into confact while the receptor protein expressed on the cell membrane of a transformant containing DNA encoding the receptor protein of the present invention by cultivating the transformant.

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20 The receptor protein used in the ligand determination of the present invention may be any protein in so far as it contains the above-described receptor protein or partial peptide of the present invention. In particular, the receptor protein expressed in a large amount by using animal cells is suitable.

For producing the receptor protein of the present invention, the above-described expression process can be used. In particular, it is preferred to carry out expression of the DNA encoding the receptor protein in cells of mammals of insects. Normally, cDNA is used as a DNA fragment encoding the desired part of the protein, but the present invention is not necessarily initial to this. For example, a gene fragment or a synthetic DNA can also be used. For transferring a DNA fragment encoding the receptor protein of the present invention into a host animal cell and expressing it efficiently. It is preferred to integrate the DNA fragment into the downstream from a polyhetrin promoter of nuclear polyhetosis virus (NPV) belonging to baculovirus whose host is insects, a promoter darived from SV40, retrovirus promoter, metallicative and qualitative assay of expression of the receptor protein can be carried out by a per se known mathod. For example, the assay can be carried out according to the method described by Nambi, P. et al., J. Blot. Chem., 257, 19555-19559 (1992).

In the ligand determination of the present invention, the receptor protein, its partial peptide or their salts may be
the receptor protein, its partial peptide or their salts as such which are purified according to a per se known method,
or a material containing the receptor protein, its partial peptide or their salts such as cells containing the receptor protein
or their membrane fractions.

In case of using the cells containing the receptor protein of the present invention, they may be immobilized with

glutaraldehyde, formalin, etc. Immobilization can be carried out by a per se known method.

The cells containing the receptor protein of the present invention are host cells expressing the receptor protein of the present invention are host cells examples of the host cells include E. coli, Bacillus subtilis, yeast, insect cells, animal cells and the like.

The cell membrane fraction is a fraction containing many cell membranes obtained by disrupting cells and then treated by a per se known method. The disruption of cells can be carried out, for example, using Potter-Ehvehlem homogenizer, Waring blender or Polytron (Kinematica), ultrasonication, French press, etc. Fractionation of the cell membrane fraction can mainty be carried out by fractionation candifulgation, density-gradient centrifugation of the like. For example, a disrupted cell suspension is centrifuged at a low rate (500 pra-3000 pm) for a short period of time (normally, about 1 minutes to and further the supernatant is centrifuged at a high rate (15000 pm-30000 pm) for about 30 minutes to about 2 hours to obtain a cell membrane fraction as precipitate. This cell membrane fraction contains the expressed receptor protein and many membrane components such as phospholipids, membrane proteins

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The amount of the receptor protein contained in the cells or the cell membrane fraction is preferably 10° to 10° molecules, more preferably 10° to 10° molecules, more preferably 10° to 10° molecules, more preferably 10° to 10° molecules are not only construction of a high sensitive screening system but also repectific activity) becomes higher, which makes not only construction of a high sensitive screening system but also

determination of a large number of samples in one lot possible.

For carrying out the above ligand determination methods (a) to (c), a suitable receptor protein fraction and a labeled test compound are required.

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The receptor protein fraction is preferably a naturally occurring receptor protein fraction or a recombinant receptor

used herein means the equivalent ligend binding activity, signal information transmission activity or the like. protein fraction having the equivalent activity to that of the naturally occurring receptor protein. The equivalent activity

noid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, ogastrin, histamine, neurotensin, TRH, pancreatic polypeptides or gallamine labeled with [4H], [125], [4C], [65S], etc. NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1α, MIP-1β, RANTES, etc.), endothelin, enterpancreastacin, prostaglandin, thromboxane, adenosine, adrenalin, α or β -chemokine (e.g., IL-8, GRO α , GRO β , GRO γ , GRO γ and related polypeptides), dopamine, motilin, armylin, bradykinin, CGRP (calcitonin gene related proteins), leukotriene, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal The labeled test compound is preferably the above-exemplified ligand such as angiotensin, bombesin, cannabi-

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fraction containing the receptor protein of the present invention are suspended in a buffer satiable for the determination beled test compound is added, is provided. The reaction is carried out at about 0°C to about 50°C, preferably about or the like. In order to minimize non-specific binding, a surfactant such as CHAPS, Tween-80™ (Kao-Atlas), digitonin, the receptor protein can be used, for example, a phosphate buffer of pH 4 to 10, preferably pH 6 to 8, Tris-HCl buffer method to prepare a receptor standard. Any buffer which does not interfere with the binding between the ligand and non-specific binding (NSB) from the total binding amount (B) exceeds 0 cpm can be selected as the ligand to the test compound labeled with [3 H], [1 25], [4 C], [3 S] or the like is added to 0.01 ml to 10 ml of the receptor suspension. suitable amount of the same buffer and the radioactivity remaining in the glass fiber filter paper is counted with a liquid scrintillation counter or a y-counter. The test compound whose count (B-NSB) obtained by subtracting the amount of pletion of the reaction, the reaction mixture was filtered through, for example, glass fiber filter paper, washed with a 4°C to about 37°C for about 20 minutes to about 24 hours, preferably about 30 minutes to about 3 hours. After com-For evaluating an amount of non-specific binding (NSB), a reaction tube, to which a large excess amount of the unla-(Peptide Kenkyu-sho), pepstatin, etc. can also be added to the buffer. A given amount (5000 cpm-500000 cpm) of the ing degradation of the receptor protein an ligand by a protease, a protease inhibitor such as PMSF, leupsplin, E-64 soxycholate, etc. and various proteins such as bovine serum albumin, gelatin, etc. to the buffer. In addition, tor inhib Specifically, for carrying out the ligand determination method of the present invention, the cells or cell membrane

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brane potential, phospholylation of intracellular protein, activation of c-los, lowering of pH, etc.) mediated by the receptor g., activity to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of intracellular Ca²* as for cAMP production inhibitory activity or the like, the activity can be delected as the production inhibitory activity contained in the cells, the assay can be carried out with addition of an inhibitor of the degradation enzyme. In addition, of an index compound of a cell stimulating activity (e.g., arachidonic acid, etc.) is difficult due to a degradation enzyme determine a product formed by a method suitable for determination of the product. Where an assay of the formation the culture is incubated for a given period of time, followed by extracting the cells or recovering the supernatant to is replaced with a fresh medium or a suitable buffer which does not have cytoloxicity. After addition of a test compound, the cells containing the receptor protein are cultivated in a well plate, etc. For ligand determination, the culture medium protein of the present invention by a known method or a commercially available determination kit. Specifically, first, formation of intracellular cAMP, formation of intracellular cGMP, production of inositol phosphate, change of cell memreceptor protein or its salt of the present invention. The above ligand determination methods (d) and (e) can be carried out by measuring a cell stimulation activity (e

of cells whose basic production is increased with forskolin, etc. septor protein of the present invention or a salt thereof, a cell membrane fraction of cells containing the receptor an essential component the receptor protein or the partial peptide of the present invention, cells containing the The determination kit of a ligand which binds to the receptor protein or its salt of the present invention comprises

protein of the present invention, or the like. Examples of the kid for ligand determination of the present invention include as follows

(1) Reagent for ligand determination

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(a) Measurement buffer and washing buffer

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is can be prepare when it is used (Gibco). The buffer is sterilized by filtration through a filter of 0.45 μm in pore diameter and then stored at 4°C or A buffer obtained by addition of 0.05% of bovine serum albumin (Sigma) to Hanks' balanced selt solution

(b) Standard of G-protein coupled receptor protein

5 x 10⁵ cells/well in a 12-well plate and cultivated at 37°C for 2 days in 5% CO_{2*}95% air to obtain a standard of the receptor protein. CHO cells expressing the receptor protein of the present invention are subjected to passage in an amount of

(c) Labeled test compound

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An aqueous solution of the labeled test compound is stored at 4°C or -20°C and, when it is used, it is diluted A test compound labeled with a commercial available [AH], [125], [14C], [35S], etc. or another suitable label.

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to 1 µM with the measurement buffer. As for a water-insoluble or slightly water-soluble compound, the compound is dissolved in dimethylformamide, DMSO, methanol, etc.

(d) Non-labeled test compound

times concentration. The same test compound as that of the labeled test compound is used to prepare a solution in 100 to 1000

(2) Measurement

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ml of the measurement buffer and then 490 μl of the buffer is added to the respective wells. (a) The receptor protein expressing CHO cells cultivated in a 12-well tissue culture plate is washed twice with 1

(c) The reaction mixture is removed and the wells are washed three times with 1 ml of the washing buffer. The (b) 5 μ l of the labeled test compound is added and reacted at room temperature for one hour scintillator A (Wako Pure Chemical Industries, Ltd.). labeled test compound bound to the cells is dissolved with 0.2 N NaOH-1% SDS and mixed with 4 mt of liquid

35 (d) Radioactivity is measured by a liquid scintillation counter (Beckman)

8 oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP in brain, pituitary, pancreas, etc. Specific examples thereof include the above-exemplified ligands, that is, angiotensin, (vasoactive intestinal and related polypeptides), dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptides or gallamine. 8, GRO¢, GROß, GRO7, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, 1-309, MIP-1¢, MIP-1β, RANTES, proteins), leukotriene, pancreastacin, prostagiandin, thromboxane, adenosine, adrenatin, lpha or eta-chemokine (e.g., L-The ligand which can bind to the receptor protein of the present invention is, for example, that specifically present

(II) Gene prophylactic and therapeutic drug of the G-protein coupled receptor protein deficiency diseases

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by deficiency of the G-protein coupled receptor protein deficiency depending upon a particular activity of the ligand. receptor protein of the present invention can be used as a drug for gene prophylaxis and therapy of diseases caused It a ligand to the receptor protein of the present invention is found out by the above method (I), DNA encoding the

ક્ષ decrease in the receptor protein of the present invention, the amount of the receptor protein in the patient's body can present invention into subject cells to express it, followed by transplantation of the cells into the patient. Then, DNA be increased to exhibit the sufficient activity of the ligand, for example, by (i) administering DNA encoding the receptor protein of the present invention to the patient to express it, or (ii) inserting DNA encoding the receptor protein of the For example, when there is a patient whose physiological activity of a ligand is scarcely expected because of

therapy of disease caused by deficiency of the receptor protein of the present invention. encoding the receptor protein of the present invention is useful as a sale and low toxic drug for gene prophytaxis and DNA encoding the receptor protein of the present invention (hereinafter sometimed abbreviated to the DNA of the

present invention) alone or, after inserted into a suitable vector such as retroviral vector, adenoviral vector, adenovirus for example, one or more pharmaceutically acceptable carriers, flavors, excipients, vehicles, preservatives, stabilizers coating, capsules, elixirs, microcapsules, etc. for oral administration, or in the form of injectable preparations such as method. For example, the DNA of the present invention can be used in the form of tablets, if necessary, providing sugar associated viral vector, etc., can be used as the above prophylactic and therapeutic drug according to a conventional aseptic solutions or suspensions in water or other pharmaceutically acceptable solutions for parenteral administration A pharmaceutical composition in a unit dosege form can be prepared by mixing the DNA of the present invention with

binders, etc. according to generally acceptable manner. The effective component is contained in the composition in such an amount that a dose in the intended desired range can be obtained. Examples of additives to be mixed in tablets, caupsels, etc. include binders such as gelatin, corn starch, tragacanth

ğ peppermint, akamono oil and cherry, and the like. In case of the capsule dosage unit form, in addition to the above acid, lubricants such as magnesium stearate, sweetenings such as sucrose, lactose and saccharin, flavors such as gum and gum arabic, excipients such as crystalline cellulose, swelling agents such as com starch, gelatin and alginic solution, for example, sesame oil, soybean oil, etc. can be used and a dissolution aid such as benzyl benzoate or glycol, polyethylene glycol), nonionic surfactants (e.g., Polysorbate 80 M HCO-50) may be further added. As an oily include physiological saline, isotonic solutions containing glucose and other adjuvants (e.g., D-sorbitol, D-mannitol water and a natural vegetable oil such as sesame oil, coconut oil, etc. Examples of the injectable aqueous solution to a conventional manner, for example, by dissolving or suspending the active component in a vehicle such as injectable component, it can contain a liquid carrier such as fat or oil. An injectable aseptic composition can be prepared according sodium chloride, etc.) and suitabla dissolution aids, for example, alcohols (e.g., ethanol), polyalcohols (e.g., propylene

benzyl alcohol, etc. can be further added

The above prophylactic and therapeutic drugs can further contain, for example, buffers (e.g., phosphate buffer, sodium acetate butfer), smoothing agents (e.g., benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g., human serum albumin, polyethylene glycol, etc.), preservatives (e.g., benzyl akohol, phenol, etc.), antioxidants, and the like. The injectable preparation thus produced is normally filled in a suitable ampoule. Since the pharmaceutical composition thus obtained is sale and low toxic, it can be administer to a human being and another mammal (e.g., rat, rabbit, sheep, pig, cattle, cat, dog, monkey, etc.). Atthough the amount of the DNA of the present invention to be in general, for oral administration to an adult human being (as 60 kg body weight), the DNA is administered in an amount of about 0.1 mg/day to about 50 mg/day, preferably about 1.0 mg/day to about 50 mg/day, more preferably about 1.0 mg to about 20 mg. For parenteral administration to an adult human being (as 60 kg body weight), it is advantageous to administer the composition in the form of an injectable preparation in an amount of about 0.01 mg/ day to about 30 mg/day, preferably about 0.1 mg/day to about 20 mg/day, more preferably about 0.1 mg/day to about 10 mg/day, though the single dosage is varied according to particular subjects, organs to be treated, symptoms, routes of administration, etc. As for other animals, the composition can be administered in the above amount with converting administered is varied according to particular subjects, organs to be treated, symptoms, roules of administration, etc., it into that for the body weight of 60 kg.

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(III) Gene diagnosing agent

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The DNA encoding the receptor protein or the partial peptide of the present invention can be used for detecting an abnormality of DNA encoding the receptor protein or the partial peptide of the present invention (abnormal gene) in a human being or another mammal (e.g., rat, rabbit, sheep, pig, cattle, cat, dog, monkey, etc.). Therefore, the DNA encoding the receptor protein or the partial peptide of the present invention is useful as a gene diagnosing agent for

(IV) Determination method of ligand to the G-protein coupled receptor protein

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The receptor protein, the partial peptide and their salts of the present invention have ligand binding properties and they can be used for determination of figand concentration in the living body in a high sensitivity.

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The method for determination of the present invention can be used, for example, in combination with a competitive method. That is, the ligand concentration can be determined by bringing a specimen into contact with the receptor protein, the partial peptide or a salt thereof of the present invention. Specifically, the determination method can be carried out, for example, according to the methods described in Hinshi Iris, Ed., "Radioimmunoassey", Kodan-sha, 1974 and "Second Series Radioimmunoassay", Kodan-sha, 1979 or their modifications.

(V) Method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein

Compounds which alter binding of a ligand to the receptor protein or its salt (e.g., peptides, proteins, non-peptide the partial peptide or their salts of the present invention, or by constructing an expression system of the recombinant receptor protein and using a receptor binding assay system utilizing the expression system. Examples of these compounds include compounds having cell stimulation activities (e.g., activity to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of intracellular Ce2*, formation of intracellular cAMP, formation of intracellular compounds, synthetic compounds, fermentation products) can be screened efficiently by using the receptor protein, cGMP, production of inositol phosphate, change of cell membrane potential, phospholylation of intracellular protein, activation of c-fos, lowering of pH, etc.) mediated by the receptor protein of the present invention (i.e., so-called agonists to the receptor protein of the present invention) and compounds which do not have such activities (i.e., so-called antagonists to the receptor protein of the present invention).

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That is, the present invention provide a method for screening for compounds which alter binding of a ligand to the receptor protein or its eatt of the present invention, or their salts which comprises comparing (i) binding of the ligand to the receptor protein or the partial peptide of the present invention or a sall thereof upon bringing the receptor protein or the partial peptide of the present invention or a salt thereol into contact with the ligand, and (ii) that upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into contact with the ligand and a

In the screening method of the present invention, an amount of the ligand bound to the receptor protein, the partial peptide or a salt thereof, a cell stimutation activity or the like is measured and compared upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into contact with the ligand, and (ii) that upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into contact with the ligand

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More specifically, the screening method of the present invention includes:

the labeled ligand, and (ii) that upon bringing the receptor protein or the partial peptide of the present invention or (a) a method for screening for compounds which alter binding of a ligand to the receptor protein or its salt of the present invention, or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount of the labeled ligand bound to the receptor protein or the partial peptide of the present invention or a salt thereof upon bringing the receptor protein or the partial peptide of the present invention or a salt thereof into contact with a salt thereof into contact with the labeled ligand and a test compound;

(b) a method for screening for compounds which alter binding of a ligand to the receptor protein or its salt of the of the labeled ligand bound to cells containing the receptor protein or a membrane fraction of the cells upon bringing the labeled ligand into contact with the cells or the membrane fraction, and (ii) that upon bringing the labeled ligand present invention, or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount and a test compound into contact with the celts or the membrane fraction;

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present invention, or their salts which comprises labeling the ligand, and measuring and comparing (i) an amount (c) a method for screening for compounds which alter binding of a ligand to the receptor protein or its salt of the of the labeled ligand bound to the receptor protein expressed on the cell membrane of a transformant containing ligand into contact with the expressed receptor protein, and (ii) that upon bringing the labeled ligand and a test DNA encoding the receptor protein of the present invention by cultivating the transformant upon bringing the labeled compound into contact with the expressed receptor protein;

(d) a method for screening for compounds which alter binding of a ligand to the receptor protein or its salt of the formation of intracellular cAMP, formation of intraceltular cGMP, production of inositol phosphate, change of cell membrane potential, phospholylation of intracellular protein, activation of c-fos, lowering of pH, etc.) mediated by the receptor upon bringing a compound which activates the receptor protein of the present invention (e.g., a ligand invention, and (ii) that upon bringing the compound which activates the receptor protein and a test compound into to the receptor protein of the present invention) into contact with cells containing the receptor protein of the present present invention, or their salts which comprises measuring and comparing (i) a cell stimulation activity (e.g., activity to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of intracellular Ca2+ contact with the cells;

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(e) a method for screening for compounds which after binding of a ligand to the receptor protein or its salt of the formation of intracellular cAMP, formation of intracellular cGMP, production of inositol phosphate, change of cell membrane potential, phospholylation of intracellular protein, activation of c-fos, lowering of pH, etc.) mediated by the receptor upon bringing a compound which activates the receptor protein of the present invention (e.g., a ligand to the receptor of the present invention) into contact with the receptor protein expressed on the cell membrane of a transformant containing DNA encoding the receptor protein by cultivating the transformant, and (ii) that upon bringing the compound which activates the receptor protein of the present invention and a test compound into present invention, or their salts which comprises measuring and comparing (i) a cell stimulation activity (e.g., activity to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of intracellular Ca2+ contact with the receptor protein expressed on the cell membrane;

agonist or antagonist, first, it is necessary to obtain candidate compounds by using cells, tissues or a cell membrane a ligand (secondary screening). When cells lissues or a cell membrane thereol are used as such, other receptor proteins example, if the human receptor protein of the present invention is used, the primary screening is not required and Before the receptor protein of the present invention is available, for screening for a G-protein coupled receptor are present, which make screening for an agonist or antagonist to the objective receptor protein difficult. However, for efficient screening for compounds which inhibit the binding of a ligand to the G-protein coupled receptor protein can be carried out. In addition, whether the compound thus screened for is an agonist or an anlagonist can be readily whether or not the candidate compounds actually inhibit the binding of human G-prolein coupled receptor protein and fraction thereof containing the G-protein coupled receptor protein of rat, etc (primary screening) and then to confirm ŝ

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The receptor protein used in the screening method of the present invention may be any protein in so far as it contains the above-described receptor protein or partial peptide of the present invention. A cell membrane fraction of the internal organs of a human being is hardly available and therefore the receptor protein expressed in a large amount a mammalian internal organ containing the receptor protein of the present invention is suitable. However, in particular, by using a recombinant is suitable for the screening.

In particular, it is preferred to carry out expression of the DNA encoding the receptor protein in cells of mammals or insects. Normally, cDNA is used as a DNA fragment encoding the desired part of the protein, but the present invention is not necessarily limited to this. For example, a gene fragment or a synthetic DNA can also be used. For transferring For producing the receptor protein of the present invention, the above described expression process can be used.

it is preferred to integrate the DNA tragment into the downstream from a polyhetrin promoter of nuclear polyhetosis talive and qualitative assay of the expressed receptor can be carried out by a per se known method. For example, the virus (NPV) belonging to baculovirus whose host is insects, a promoter derived from SV40, retrovirus promoter, meta DNA tragment encoding the receptor protein of the present invention into a host animal cell and expressing it efficiently, assey can be carried out according to the method described by Nambi, P. et al., J. Biol. Chem., 267, 19555-19559 allothionein promoter, human hoat shock promoter, cytomegalovirus promoter, SRa promoter or the like. The quanti-

receptor protein, its partial peptide or the salt as such which is purified according to a per se known method, or a material containing the receptor protein, its partial peptide or their selts such as cells containing the receptor protein In the screening method of the present invention, the receptor protein, its partial peptide or their salts may be the

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or their membrane fractions In case of using the cells containing the receptor protein of the present invention, they may be immobilized with

glutaraldehyde, formalin, etc. Immobilization can be carried out by a per se known method. μe present invention. Preterrad examples of the host cells include <u>Ε</u>, <u>coli, Bacillus subtilis,</u> yeast, insect cells, animal The cells containing the receptor protein of the present invention are host cells expressing the receptor protein of

ation of the cell membrane fraction can mainly be carried out by fractionation centrifugation, density-gradient centriftreated by a per se known method. The disruption of cells can be carried out, for example, using Potter-Eivehjem homogenizer, Waring blender or Polytron (manufactured by Kinematica), ultrasonication, French press, etc. Fractionfraction contains the expressed receptor protein and many membrane components such as phospholipids, membrane 30000 rpm) for about 30 minutes to about 2 hours to obtain a cell membrane fraction as a precipitate. This cell membrane period of time (normally, about 1-10 minutes) and further the supernatant is centrifuged at a high rate (15000 rpmugation or the like. For example, a disrupted cell suspension is centriluged at a low rate (500 rpm-3000 rpm) for a short alls and the like. The cell membrane fraction is a fraction containing many cell membranes obtained by disrupting cells and then

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is higher, a ligand binding activity (specific activity) becomes higher, which makes not only construction of a high senfraction is preferably 10³ to 10⁸ molecules, more preferably 10⁵ to 10⁷ molecules per one cell. As the expression level proteins and the like. The amount of the receptor protein contained in the cells containing the receptor protein or the cell membrane

မွ compound are required. The receptor protein fraction is preferably a naturally occurring receptor protein fraction or a The equivalent activity used herain means equivalent ligand binding activity, signal information transmission activity recombinant receptor protein fraction having the equivalent activity to that of the naturally occurring receptor protein silive screening system but also determination of a large number of samples in one lot possible. For carrying out the above screening methods (a) to (c), a suitable receptor protein fraction and a labeled test

છ The labeled ligand includes labeled ligands and labeled ligand analog compounds. For example, the ligand labeled

a receptor protein standard. Any buffer which does not interfere with the binding between the ligand and the receptor containing the receptor protein of the present invention are suspended in a buffer satiable for the screening to prepare with [3H], [125]], [14C], [35S], etc. can be used. by Peplide Kenkyu-sho), pepsialin, etc. can also be added to the buffer. A given amount of the labeled ligand (5000 protein can be used, for example, a phosphate buffer of pH 4 to 10, preferably pH 6 to 8, Tris-HCl buffer or the like. In 🖏 the receptor protein and the ligand by a protease, a protease inhibitor such as PMSF, leupeptin, E-64 (manutactured yder to minimize non-specific binding, a surfactant such as CHAPS, Tween-80™ (Kao-Atlas), digitonin, deoxycholate, is, and various proteins such as bovine serum albumin, gelatin, etc. to the buffer. In addition, for inhibiting degradation Specifically, for carrying out the screening method of the present invention, the cells or cell membrane fraction

ŝ 8 a glass fiber filter paper, washed with a suitable amount of the same buffer and the radioactivity remaining in the glass about 0°C to about 50°C, preferably about 4°C to about 37°C for about 20 minutes to about 24 hours, preferably about a test compound is present in the receptor suspension. For evaluating an amount of non-specific binding (NSB), a cpm-500000 cpm) is added to 0.01 ml to 10 ml of the receptor suspension and, at the same time, 10⁻⁴ M to 10⁻¹⁰ M of taking the count (B₀-NSB) obtained by subtracting the count of non-specific binding (NSB) from the count (B₀) obtained count (B-NSB) is 50% or lower can be selected as a candidate compound capable of inhibiting antagonism, when 30 minutes to about 3 hours. After completion of the reaction, the reaction mixture was filtered through, for example reaction tube to which a large excess amount of the unlabeled ligand is added is provided. The reaction is carried out fiber filter paper is counted with a liquid scintillation counter or a y-counter. The test compound whose specific binding

to enhance or inhibit release of arachidonic acid, release of acetyl choline, release of intracellular Ca2*, formation of phospholylation of intracellular protein, activation of c-tos, lowering of pH, etc.) mediated by the receptor protein of the intracellular cAMP, formation of intracellular cGMP, production of inositol phosphate, change of cell membrane potential without any antagonistic material as 100%. The above screening methods (d) and (e) can be carried out by measuring a cell stimulation activity (e.g., activity

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contained in the cells, the assay can be carried out with addition of an inhibitor of the degradation enzyme. In addition of an index compound of a cell stimulating activity (e.g., arachidonic acid, etc.) is difficult due to a degradation enzyme determine a product formed by a method suitable for determination of the product. Where an assay of the formation the culture is incubated for a given period of time, followed by extracting the cells or recovering the supernatant to replaced with a tresh medium or a suitable buffer which does not have cytoloxicity. After addition of a test compound, taining the receptor protein are cultivated in a multi-well plate, etc. For carrying out screening, the culture medium is present invention by a known method or a commercially available determination kit. Specifically, first, the cells conas for cAMP production inhibitory activity or the like, the activity can be detected as the production inhibitory activity

ö of cells whose basic production has been increased with forskolin, etc. protein are required. As cells expressing the receptor protein of the present invention, for example, naturally occurring cell strains containing the receptor protein of the present invention or the above-described recombinants expressing For carrying out the screening by measurement of a cell stimulating activity, cells expressing a suitable receptor

extracts, vegetable extracts, animal tissue extracts and the like and these compounds may be novel compounds or the receptor protein are preferred. Examples of the test compounds include peptides, proteins, non-peptide compounds, synthetic compounds, cell

a salt thereol, cells containing the receptor protein of the present invention, a cell membrane fraction of cells containing invention comprises as an essential component the receptor protein or the partial peptide of the present invention or known compounds. The kit for screening for a compound which alters binding of a ligand to the receptor protein or its salt of the present

8 the receptor protein of the present invention, or the like. Examples of the screening kit of the present invention include as follows

(1) Reagent for screening

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(Gibco). The buffer is sterilized by filtration through a filter of 0.45 μm in pore diameter and then stored at 4°C or (a) Measurement buffer and washing buffer is can be prepare when it is used A buffer obtained by addition of 0.05% of bovine serum albumin (Sigma) to Hanks' balanced selt solution

(b) Standard of G-protein coupled receptor protein

 5×10^5 cells/well in a 12-well plate and cultivated at 37°C for 2 days in 5% CO $_2$ -95% air to obtain a standard of CHO cells expressing the receptor protein of the present invention are subjected to passage in an amount of

(c) Labeled ligand

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An aqueous solution of the labeled test compound is stored at 4°C or -20°C and, when it is used, it is diluted A ligand labeled with a commercial available [3H], [125], [14C], [35S], etc. or another suitable label

to 1 µM with the measurement buffer.

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mM and stored at -20°C. (d) Ligand standard solution A ligand is dissolved in PBS containing 0.1% bovine serum albumin (Sigma) at the final concentration of 1

(2) Measurement

ന്നി of the measurement buffer and then 490 µl of the same buffer is added to the respective wells. (a) The receptor protein expressing CHO cells cultivated in a 12-well tissue culture plate is washed twice with 1

Ġ, (b) 5 μ l of a 10-3 to 10-10 M solution of a test compound is added and then 5 μ l of the labeled ligand is added. They are reacted at room temperature for one hour. In order to evaluate a non-specific binding amount, 5 µl 10-3 M ligand is added.

labeled ligand bound to the cells is dissolved with 0.2 N NaOH-1% SDS and mixed with 4 ml of liquid scintillator (c) The reaction mixture is removed and the wells are washed three times with 1 ml of the washing buffer. The

50 (d) Radioactivity is measured by a liquid scintillation counter (Beckman). Percent Maximum Binding (PMB) is calculated by the equation [1]: A (Wako Pure Chemical Industries, Ltd.).

PMB=[(B-NSB)/(B₀-NSB)] × 100

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non-specific binding, and B_0 is a maximum binding value. wherein PMB is Percent Maximum Binding. B is a value obtained with addition of a specimen, NSB is a value of

The compound or its salt obtained by using the screening method of the screening kit of the present invention alters binding of a ligand to the receptor protein or its salt of the present invention. Specifically, it is a compound or a sall thereof which binds to the receptor protein of the present invention and a exhibits cell stimulating activity mediated by the receptor protein (i.e., so-called an agonist to the receptor protein of the present invention) or which binds to the receptor protein of the present invention but does not exhibit the cell stimulating activity (i.e., so-called an anlagonist of the present invention).

Examples of the compounds include peptides, proteins, non-peptide compounds, synthetic compounds, fermented

Since the agonist to the receptor protein of the present invention has the same physiological activity as that of a ligand to the receptor protein, it is useful as an active component of a safe and low toxic pharmaceutical composition products and the like and they may be novel compounds or known compounds.

On the other hand, since the antagonist to the receptor protein of the present invention can inhibit the physiological having the ligand activity.

activity of a figand to the receptor protein, it is useful as an active component of a sale and low toxic pharmaceutical composition for inhibiting the ligand activity.

If the compound or its salt obtained by the screening method or the screening kit of the present invention is used for a pharmaceutical composition as described above, any conventional manner can be employed. For example, the compound or its salt of the present invention can be used in the form of tablets, if necessary, providing sugar coating, capsules, elikirs, microcapsules, etc. for oral administration, or in the form of injectable preparations such as aseptic solutions or suspensions in water or other pharmaceutically acceptable solutions for parenteral administration. A pharmaceutical composition in a unit dosage form can be prepared by mixing the compound or its salt of the present invention with, for example, one or more pharmaceutically acceptable carriers, flavors, excipients, vehicles, presenvatives, stabilizers, binders, etc. according to generally acceptable manner. The effective component is contained in the composition in such an amount that a dose in the intended desired range can be obtained.

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Examples of additives to be mixed in tablets, caupsets, etc. include binders such as gelatin, com starch, tragacanth gum and gum arabic, excipients such as crystalline cellulose, swelling agents such as com starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweetenings such as sucrose, lactose and saccharin, flavors such as peppermint, akamono oil and cherry, and the like. In case of a capsule dosage unit form, in addition to the above components, it can contain a liquid carrier such as fat or oil. An injectable aseptic composition can be prepared according to a conventional manner, for example, by dissolving or suspending the active component in a vehicle such as injectable water and a natural vegetable oil such as sesame oil, coconut oil, etc. Examples of injectable aqueous solution include physiological saline, isotonic solutions containing glucose and other adjuvants (e.g., D-sorbitol, D-mannitol, sodium chloride, etc.) and suitable dissolution aids, for example, alcohols (e.g., ethanol), polyalcohols (e.g., As an oily solution, for example, sesame oil, soybean oil, etc. can be used and a dissolution aid such as benzyl benzoate propylene glycol, polyeitrylene glycol), nonionic surfaciants (e.g., Polysorbaie 80™, HCO-50) may be further added or benzyl alcohol, etc. can be further added. The composition can further contain, for example, buffers (e.g., phosphate buller, sodium acetate buller), smoothing agents (e.g., benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g., human serum albumin, polyethylene glycol, etc.), preservatives (e.g., benzyl alcohol, phenol, etc.), antioxidants, and the like. The injectable preparation thus produced is normally filled in an appropriate ampoule. 52 8

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Since the pharmaceutical composition thus obtained is safe and low loxic, it can be administer to a human being and another mammal (e.g., rat, rabbit, sheep, pig, cattle, cat, dog, monkey, etc.)

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Although the amount of the compound or its salt of the present invention to be administered is varied according to particular subjects, internal organs to be treated, symptoms, routes of administration, etc. in general, for oral administration to an adult human being (as 60 kg body weight), the DNA is administered in an amount of about 0.1 mgday to about 100 mg/day, prelerably about 1.0 mg/day to about 50 mg/day, more preferably about 1.0 mg to about 20 mg. For parenteral administration to an adult human being (as 60 kg body weight), it is advantageous to administer the composition in the form of an injectable preparation in an amount of about 0.01 mg/day to about 30 mg/day, preferably about 0.1 mg/day to about 20 mg/day, more preferably about 0.1 mg/day to about 10 mg/day, though the single dosage is varied according to particular subjects, internal organs to be treated, symptoms, routes of administration, etc. As for other animals, the composition can be administered in the above amount with converting it into that for the body weight

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(VI) Preparation of antibody or antiserum against the G-protein coupled receptor protein, the partial peptide or salts

peptide or their salts of the present invention can be prepared by using the receptor protein or the partial peptide of the present invention or a salt thereof as the antigen according to a conventional antibody or antiserum preparation An antibody (e.g., monoclonal antibody, polyclonal antibody) or antiserum against the receptor protein, the partial process. For example, a monoclonal antibody can be prepared as follows.

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Preparation of monoclonal antibody

(a) Preparation of monoclonal antibody producer cells

viated to the receptor protein, etc.) as such or together with a suitable carrier or diluent is administered to a site of a The receptor protein or the partial peptide of the present invention or a sall thereof (hereinalter sometimes abbremammal which permits the antibody production. For enhancing the antibody production capability, complete Freund's adjuvant or incomplete Freund's adjuvant may be administered. Normally, the receptor, etc. is administered once every 3 weeks to 6 weeks, in total, about 2 to about 10 times. The mammal to be used include monkey, rabbit, dog, guinea plg, mouse, rat, sheep, goat, chicken and the like and mouse or rat is preferred. 2

lected from warm blood animals immunized with the antigen, for example, rat and, 2 days to 5 days after the final anliserum can be carried out, for example, by reacting the labeled receptor protein, etc. as described hereinatter and For preparing monoclonal antibody producer cells, an individual whose antibody titer has been confirmed is seimmunization, its spleen or tymph node is collected and antibody producer cells contained therein are fused with myeloma cells to prepare the desired monoclonal antibody producer hybridoma. Measurement of the antibody titer in an an antiserum and then measuring the activity of the labeling agent bound to the antibody. The cell fusion can be carried oul according to a known method, for example, the method described by Koehler and Milstein, Nature, 256, 495 (1975). As a fusion promoter, for example, polyethylene glycol (PEG) or Sendai virus (HVJ), preferably PEG can be used 55

Examples of myeloma cells include NS-1, P3U1, SP2/0, AP-1 and the like and P3U1 is preferred. The proportion of the number of antibody producer cells (spleen cells) and the number of myeloma cells to be used is preferably about Cell fusion can be carried out efficiently by incubating a mixture of both cells at about 20°C to about 40°C, preferably l : 1 to about 20 : 1 and PEG (preferably PEG 1000-PEG 6000) is added in concentration of about 10% to about 80% about 30°C to about 37°C for about 1 minute to about 10 minutes. 20

Various methods can be used for screening for a hybridoma producing the antibody against the receptor protein, etc. For example, there may be mentioned a method wherein a supernatant of the hybridoma is added to a solid phase (e.g., microplate) to which the receptor protein antibody is adsorbed directly or together with a carrier and then an antimmunoglobulin antibody (if cells of a mouse is used in cell fusion, anti-mouse immunoglobulin antibody is used) of Protein A labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the receptor protein, etc. bound to the solid phase, and a method wherein a supernalant of the hybridoma is added to a labeled with a radioactive substance or an enzyme is added to detect the monoclonal antibody against the receptor solid phase to which an anti-immunoglobutin antibody or Protein A is adsorbed and then the receptor protein, etc., protein, etc. bound to the solid phase. 52 3

Normally, a medium for animal cells to which HAT (hypoxanthine, aminopterin, trymidine) are added is employed. Any Selection of the monoclonal antibody can be carried out according to a per se known method or its modification. selection and growth medium can be employed in so far as the hybridoma can grow. For example, RPMI 1640 medium containing 1% to 20%, preferably 10% to 20% fetal bovine serum, GIT medium containing 1% to 10% fetal bovine serum (Wako Pure Chemical Industries, Ltd.), a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Selyaku) and the like can be used. Normally, the cultivation is carried out at 20°C to 40°C, preferably 37°C for about 5 days to about 3 weeks, preferably 1 week to 2 weeks under about 5% CO₂ gas. The antibody titer of the supernatant of a hybridoma culture can be measured according to the same manner as described above with respect to the antibody titer of the anti-G-protein coupled receptor in the antiserum. ક્ષ

(b) Purification of monoclonal antibody

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Separation and purification of a monoclonal antibody against the receptor protein, etc. (hereinatter sometimes referred to as the anti-receptor protein monoclonal antibody) can be carried out according the same manner as those alcoholic precipitation, isoelectric point precipitation, electrophoresis, adsorption and desorption with ion exchangers (e.g., DEAE), ultracentrilugation, gel filtration, or a specific purification method wherein only an antibody is collected with an active adsorbent such as an antigen-binding solid phase, Protein A or Protein G and dissociating the binding of conventional polyclonal antibodies such as separation and purification of immunoglobulins, for example, salting-out, to obtain the antibody. 20

The antibody against the receptor protein, etc. of the present invention prepared by the above (a) and (b) can specifically recognize the receptor protein, etc. of the present invention and therefore it can be used in a quantitative determination of the receptor protein, etc. of the present invention in a specimen, particularly, a quantitative determination by a sandwich immunoassay. That is, the present invention also provides:

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(a) a method for determination of the receptor protein or its salt of the present invention in a specimen fluid which comprises reacting an antibody against the receptor protein or the partial peptide of the present invention, the

being that recognizing the N-terminal of the receptor protein of the present invention and the other antibody being or continuously, and then measuring the activity of the labeling agent on the insolubilized carrier, one antibody comprises reacting the specimen fluid, an antibody insolubilized on a carrier and a labeled antibody simultaneously (b) a method for determination of the receptor protein or its salt of the present invention in a specimen fluid which proportion of the labeled receptor protein or the labeled partial peptide bound to the antibody; and specimen fluid and the labeled receptor protein or the labeled partial peptide competitively and measuring the

that reacting with the C-terminal of the receptor protein of the present invention.

monoclonal antibody recognizing the receptor protein of the present invention, etc. can be used for detection by his tological stains and the like. For these purposes, the antibody molecular as such can be used or F(ab)2. Fab' or Fab prolein, etc. of the present invention is not specifically limited and any determination method can be used in so far as fraction of the antibody molecule can also be used. A method for determination using an antibody against the receptor the like, a sandwich method as described hereinafter is preferred. an amount of an antigen, antibody or antibody-antigen corresponding to an amount of an antigen in a fluid to be deimmunometirc method and sandwich method are suitably employed. In particular, in view of sensitivity, specificity and sing standard solutions containing known amounts of the antigen. For example, nephelometry, competitive method, ırmined can be delected by a chemical or physical means and calculated based on a calibration curve prepared by In addition to the determination of the receptor protein or its salt of the present invention, the anti-receptor protein

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25 8 the like. As the above enzymes, that having good stability and high specific activities is preferred and, for example, materials, luminous materials and the like can be used. Examples of radioisotopes include [125], [131], [4H], [4C] and materials, there are luminol, luminol derivatives, luciferin, lucigenin and the like. In addition, biotin-avidin system can the fluorescent materials, for example, there are fluorescamine, fluorescein isothiccyanate and the like. As the luminous there are β-galactosidase, β-glucosidase, alkaline phosphatase, peroxidase, malate dehydrogenase and the like. As be used for binding of an antibody or antigen to a labeling agent. As a labeling agent used in a determination method using a labeled reagent, radioisotopes, enzymes, fluorescen

chemical bond for insolubilizing or immobilizing a protein or an enzyme can be used. Examples of the carrier include insoluble polysaccharides such as egarose, dextran, cellulose and the like, synthetic resins such as polystyrene, poly-For insolubilization of an antigen or antibody, physical adsorption can be used or, normally, a method using a

딿 antibody or labeled antibody is not necessary one kind of antibodies and, in order to improve measuring sensitivity, to this method. In addition, in an immunoassay by a sandwich method, an antibody to be used as the solid phase in the specimen. The order of the primary and secondary reactions can be reversed and they can be carried out uring the labeling agent on the insoluble carrier to determine the amount of the receptor protein of the present invention (primary reaction) and further reacting a labeled anti-receptor protein antibody (secondary reaction), followed by measacrylamide, silicone and the like, glass and the like. etc., a mixture of two or more kinds of antibodies can be used. simultaneously or separately at different times. The above-described labeling agent and insolubilization can be applied In a sandwich method, a specimen fluid to be tested is reacted with an insolublized anti-receptor protein antibody

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preferably, the antibodies against the receptor protein, etc. used in the primary and secondary reactions are those naving different binding sites for the receptor protein. For example, when the antibody used in the secondary reaction cognizing an region other than the C-terminal region, for example, the N-terminal region. that recognizing the C-terminal region of the receptor protein, the antibodies used in the primary reaction is that In the method for determination of the receptor protein, etc. by the sandwich method of the present invention

In a competitive method, an antigen in a specimen fluid and a labeled antigen are reacted with the antibody competitively other than a sandwich method, for example, a competitive method, immunometric method, nephelometry and the like or a soluble antibody is used as the first antibody and an immobilized solid phase antibody is used as the second against the above antibody. In the solid phase method, an immobilized solid phase antibody is used as the first antibody antibody is used as the antibody and B/F separation can be carried out by using polyethylene glycol, a second antibody reaction, both liquid phase method and solid phase method can be employed. In the liquid phase method, a soluble the amount of the labeling agent of either B or F to determine the amount of the antigen in the specimen fluid. In this and, after separation of the unreacted tabeled antigen (F) from the labeled antigen bound to the antibody (B), measuring The antibody against the receptor protein, etc. of the present invention can also be used for a measuring system

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antibody and an immobilized solid phase antigen is added to permit the unreacted labeled antibody to bind to the solid liquid phase. Alternatively, an antigen in a specimen fluid to be tested is reacted with an excess amount of a labeled either phase is measured to determine the amount of the antigen in the specimen fluid. phase, followed by the separation of the solid phase from the liquid phase. Then, the amount of the labeling agent of are reacted with a give amount of a labeled antibody, competitively and then the solid phase is separated from the In the immunometric method, an antigen in a specimen fluid to be tested and an immobilized solid phase antigen

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amount of a precipitate formed is small, laser nephelometry wherein diffusion of laser is utilized can be suitably em precipitate formed is measured. Even when the amount of an antigen in a specimen fluid to be tested is small and the In nephelometry, an antigen-antibody reaction is carried out in a get or solution and the amount of an insoluble

ŏ 3 Eizi Ishikawa et al., Third Series, Enzyme Immunoassey, Igaku-shoin (1987); Method in Enzymology, Vol. 70, Immusha (1974); Hiroshi irie, Ed, Second Series, Radioimmunoassey, Kodan-sha (1979); Elzi Ishikawa et at., Ed., Enzyme methods together with conventional artisan's technical consideration. As for details of these general technical means, or its salt of the present invention can be constructed based on conventional conditions and procedures in respective special conditions, procedures and the like is not required. That is, the determination system of the receptor protein ibid., Vol. 92, Immunochemical Techniques (Part E. Monoclonal Antibodies and General Immunoassay Methods); ibid. nochemical Techniques (Part A)), Academic Press; ibid., Vol. 73, Immunochemical Techniques (Part B); ibid., Vol. 74, Immunoassay, Igaku-shoin (1978); Eizi Ishikawa et al., Ed., Second Series, Enzyme Immunoassay, Igaku-shoin (1982); reference can be made to various reviews, texts and the like, for example, Hiroshi Irie, Ed., Padioimmunoassay, Kodan Immunochemical Techniques (Part C); ibid., Vol. 84, Immunochemical Techniques (Part D: Selected Immunoassays) When employing these immunoassay methods in the determination method of the present invention, to set any

Vol. 121, Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies and the like sitivity by using an antibody against the receptor protein, etc. of the present invention. As described hereinabove, the receptor protein or its salt of the present invention can be determined at high sen-

8 IUPAC-IUB Commission on Biochemical Nomenclature or those conventionally used in the art. The examples are as follows. When the amino acid has an optical isomer, the amino acid is L-isomer unless otherwise stated. In the specification and drawings, the abbreviations of bases, amino acids and the like are those according to

25 છ છ 55 50 dTTP. DATP mRNA cDNA: dGTP ATP: dCTP IZA Ē SDS: EDTA = Val: Ala Glu: Cys 1 Fen: ¥.: Pie Ag Уs Asp guanine thymine complementary deoxyribonucleic acid messenger ribonucleic acid deaxyribonucleic acid deoxyadenosine triphosphate cytosine glycine sodium dodecylsulfate deoxycytidine triphosphate deoxyguanosine triphosphate deoxythymidine triphosphate ribonucleic acid cysteine threonine isoleucine leucine valine alanine enzyme immunoassay ethylenediaminetetraacetic acid adenosine triphosphate asparagine glutamine lysine glutamic acid serine proline tyrosine aspartic acid methionine tryptophan phenylalanine histidine arginine

pyroglutamic acid pGlu: ... B

methyl group ethyl group

butyl group .. B

thiazolidin-4(R)-carboxamide group phenyl group 윤 2

The sequences in the Sequence Listing of the present specification are as follows.

SEQ ID NO: 1 5

This represents an amino acid sequence of the G-protein coupled receptor protein derived from a human brain.

SEQ ID NO: 2

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This represents an amino acid sequence of the G-protein coupled receptor protein derived from a human brain, which is a variant of SEQ ID NO: 1 having additional 61 amino acids at the N-terminal thereof.

SEQ ID NO: 3

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This represents a nucleotide sequence of DNA encoding the G-protein coupled receptor protein derived from a human brain having the amino acid sequence represented by SEQ ID NO: 1.

SEC ID NO: 4

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This represents a nucleotide sequence of DNA encoding the G-protein coupled receptor protein derived from a human brain having the amino acid sequence represented by SEQ ID NO: 2, which is a variant of SEQ ID NO: 3 having additional 183 bases at the 5'-terminal thereof.

SEQ ID NO: 5 8

A nucleotide sequence of EST which has been registered with a data base (NCB) abEST) under the accession number of T08099.

SEO ID NO: 6 ક્ષ

A nucleotide sequence of EST which has been registered with a data base (NCBI abEST) under the accession umber of T27053.

SEQ ID NO: 7 \$

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEQ ID NO: 8 â

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEC ID NO: 9 જ

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein

coupled receptor protein of the present invention.

SEQ ID NO: 10

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This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

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SEQ ID NO: 11

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEQ ID NO: 12

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEQ ID NO: 13

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This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein

coupled receptor protein of the present invention.

SEQ ID NO: 14

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This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein coupled receptor protein of the present invention.

SEQ ID NO: 15

This represents a nucleotide sequence of a synthetic DNA used in screening for cDNA encoding the G-protein

National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science & Technology Ministry of International Trade & Industry (1-3. Higasi 1-chome, Taukubashii Ibaraki, 305 Japan) according to the Budapast Treaty under the accession number of FERM BP-5724 since October 25, 1996 and also deposited with Institute for The transformant Escherichia coli HB101/pHEBF2 obtained in Example 1 hereinafter has been deposited with coupled receptor protein of the present invention. 52

The following examples further illustrate the present invention in detail but are not to be construed to limit the scope of IFO 16044 since October 21, 1996. thereof.

Fermentation Osaka (IFO, 17-85, Juso-honmachi 2-chome Yodogawa-ku, Osaka, 532 Japan) under accession number

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Example 1

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Obtaining cDNA of entire translated region of receptor protein from human poly(A)+RNA and sequence analysis of its nucleotide sequence

In order to obtain cDNA of the translated region about the C-terminal of the receptor protein encoded by the known nucleotide sequences, accession numbers of T08099 (SEQ ID NO: 5) and T27053 (SEQ ID NO: 6), 3°FACE (1) Obtaining cDNA of translated region about C-terminal of the receptor protein from human fetal brain poly (A)*RBNA by 3' RACE (Rapid Amplification of cDNA End) method and sequence analysis of its nucleotide sequence

First, the following two primers were synthesized based on the known nucleotide sequences. method was carried out by using human fetal brain poly(A)+RNA as a PCR template.

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B1: (SEQ ID NO: 7)

5'-AAGTTGGCTGTCATCTGGGTGGGCTC-3'

B2: (SEQ ID NO: 8)

5'-reagercetgergregeageregeage-3'

Then, a PCR template of 3PACE method was prepared from 1 µg human latal poly(A)*RNA (Clontech) by using 3'BACE kit (Gibco BRL). The first PCR was carried out using the primer attached to 3'RACE kit and B1 primer. The conditions were 30 seconds at 95°C, 60 seconds at 65°C and 180 seconds at 72°C, for 35 cycles and

cloning kit (Invitrogen) and transferred into E. coli JM109. As a result of sequence analysis, it was found that the μl of the first PCR mixture as the template under the same conditions for 35 cycles except that B2 primer was used Ex Taq (Takara Shuzo) was used as the DNA polymerase. The second PCR reaction was carried out by using 1 (2) Obtaining cDNA of translated region about N-terminal of the receptor protein from human fetal brain poly amplified band had the C-terminal region of the above-described known nucleotide sequences. instead of B1 primer. After electrophoresis, a band formed of 1.5 kb was recovered, subcloned by using a TA

(A)+RNA by 5'RACE method (marathon method) and sequence analysis of its nucleotide sequence above-described known nucleotide sequences, 5'RACE method was carried out by using human fetal brain poly In order to obtain cDNA of the translated region about the N-terminal of the receptor protein encoded by the

(A)+ RNA as a PCR template. First, the following two primers were synthesized based on the above-described known nucleotide sequences

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B9: (SEQ ID NO: 10 5'-CATGCGGGCGTTCTGGTAGGTCATCAC-3' B8: (SEQ ID NO: 9)

cording to the manual of SPACE kit (Clontech). The first PCR was carried out using the primer attached to SPACE by the nucleotide sequence of from the 625th base (ATG; Met) to the 2067th base (TGC; Cys) as shown by Fig. (1). The presence of the 7-transmembrane receptor protein comprising 481 amino acids (SEQ ID NO: 1) encoded a TA cloning kit (Invitrogen), and sequence analysis was carried out according to the same manner as in the above instead of B9 primer. After electrophoresis, a band formed of about 1 kb was recovered and subcloned by using 50-fold dilution of the first PCR mixture as the template under the same conditions except that B8 primer was used 98°C and 180 seconds at 70°C, for 5 cycles; and 10 seconds at 98°C and 180 seconds at 88°C, for 35 cycles. Ex kit and B9 primer. The conditions were 10 seconds at 98°C and 180 seconds at 72°C, for 5 cycles; 10 seconds at Taq (Takara Shuzo) was used as the DNA polymerase. The second PCR reaction was carried out by using 1 µl of has been confirmed based on the results of the above (1) and (2). Fig. 2 shows the result of hydrophobic plotting . Then, a PCR template of S'RACE method was prepared from 1 µg human tatat poly(A)+RNA (Clontech) ac-

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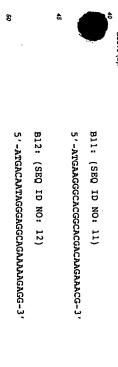
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5'-GAAGAGGATGGGCAGGCAGAAGTAGCAG-3'

of this amino acid sequence. (3) The N-terminal side of the above receptor protein was further examined and 5'HACE method at the 5'side was further carried out to determine a transcription initiation codon.

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above (2) The following two primers were synthesized based on the translation region of the receptor protein obtained in the



primer B12 instead of the primer B8, respectively to amplify the templates derived from the poly(A)+RNAs of the above as described in the above (2), twice PCR were carried out by using the primer B11 instead of the primer B9 and the brain poly(A)+RNA (Clontech) or human cerebellum poly(A)+RNA (Nippon gene). Then, according to the same manner and then subjected to sequence analysis. internal organs. After electrophoresis of the reaction product, a band formed was recovered, subcloned by TA cloning According to the same manner as in the above (2), a PCR template was prepared by using the above human fetal

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As a result, the sequence obtained by combining the above (1) to (3) had the nucleotide sequence (SEQ ID NO

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encoded the 7-transmembrane receptor protein comprising 542 amino acids (SEQ ID: NO 2). The result of the hydro-4) of from the 442nd base (ATC; Met) to the 2067th base (TCG; Cys) as shown in Fig. 3 and was confirmed that it phobic plotting of this sequence is shown in Fig. 4

The following primers were synthesized based on this sequence.

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5'-GTCGACGAGATGTGTGAGGGCAGCAAAGAGTGC-3' HEF: (SEQ ID NO: 13)

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5'-TACTGGGGCCTCAGCAAGGTGTGCCCAG-3'

HER-1: (SEQ ID NO: 14)

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8 E. coll HB101 to obtain the transformant, E. coll HB101/pHEBF2. The DNA contained in the plasmid pHEBF2 has the fetal brain cDNA library. After subcloning in E. coli, a clone without any PCR error was selected and transformed into nucleotide sequence represented by SEQ ID NO: 4 (Fig. 3) and the nucleotide sequence represented by SEQ ID NO: PCR was carried out by using these two primers to amplify the coding region for the receptor protein from human

seconds at 68°C to amplify the coding region, the cDNA of the receptor protein as shown by Fig. 3 was obtained from 3 is contained therein. When PCH was carried out by using the above two primers under the conditions of 30 seconds at 95°C and 90

25 In order to obtain the cDNA of the receptor protein form adult brain, HEF-2 primer was synthesized instead of HEF

fetal brain

HEF-2: (SEQ ID NO: 15)

5'-GTCGACTGGCTGTCTCCTGCTCATCCAGCCAT-3'

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receptor protein shown in Fig. 1 was obtained. The N-terminal of this receptor protein was shorter than that of the receptor protein shown in Fig. 3 by 61 amino acids. When adult brain poly(A)+RNA was amplified by using the primers HEF-2 and HEF-1, the cDNA encoding the

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8 brain. On the other hand, cDNA encoding the receptor protein shown by Fig. 3 is obtained from fetal brain and the presence of the long chain receptor protein shown by Fig. 3 has also been confirmed. In view of these results, it has been found that the receptor protein shown by Fig. 1 is predominantly expressed in adult initiation of translation just before the translation initiation codon and a defined signal sequence in the N-terminal region. The receptor protein shown in Fig. 1 has a consensus sequence called as Cossack sequence which indicates

Example 2

Confirmation of expression specificity in various tissues

ŝ the plasmid pHEBF2 obtained in Example 1 as a probe Northern blot was carried out by using cDNA encoding the receptor protein of the present invention contained in

of the filter to light was made as -80°C for one week. As shown in Fig. 5, it has been found that this receptor protein ization was carried out by using human MTN Blot (Clontech) and according to the manual attached thereto. Exposure mRNA is specifically expressed in brain. The cDNA was labeled with Amarsham's multiprime kit and [22P]dCTP according to the manual of the kit. Hybrid

8 છ coding them of the present invention can be used (a) for determination of ligands, (b) for obtaining antibodies and of drug design based on comparison of structurally analogous ligand receptors, (1) as reagants for preparing probes in gene diagnoses, PCR primers, etc., (g) drugs for gene prophylactic and therapy, and the like. binding assay systems and screening for drug candidate compounds by using the expression systems, (e) for practice antisera, (c) for construction of recombinant receptor protein expression systems, (d) for development of receptor As described hereinabove, the G-protein coupled receptor protein, its partial peptide or their satts and DNAs en

SEQUENCE LISTING

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		(2) INFORMATION FOR SEO ID NO: 1
(1) GENERAL INFORMATION	ક	(i) SEQUENCE CHARACTERISTICS:
(i) APPLICANT: Takeda Chemical Industries Ltd		(A) LENGTH: 401 (B) TYPE: amino acid
(ii) TITLE OF THE INVENTION: Novel Human G-Protein Coupled Receptor Protein and its DNA	ōî	(D) TOPOLOGY: linear
(iii) NUMBER OF SEQUENCES: 15		(ii) MOLECULAR TYPE: peptide
(iv) CODDECDONIDENCE A PROFESS.	21	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual Property (B) STREET: Two New Horizons Court		Met Arg Trp Leu Trp Pro Leu Ala Val Scr Leu Ala Val Ile Leu Ala I 5 10 15
(C) CITY: Brentford	20	Val Giv I on Ser Arr Val Ser Glv Gly Box I on His I on Gly Ace
(E) COUNTRY! United Kingdom		20 25 30
(r) rost code: 1 w8 yEP	25	His Arg Ala Glu Thr Gln Glu Gln Gln Ser Arg Ser Lys Arg Gly Thr
(v) COMPUTER READABLE FORM:		35 40 45
(A) MEDIUM I TPE: Diskette (B) COMPUTER: IBM Compatible	30	Glu Asp Glu Glu Ala Lys Gly Val Gln Gln Tyr Val Pro Glu Glu Trp 50 55 60
(vi) CURRENT APPLICATON DATA:		
(A) APPLICATION NUMBER: (B) FILING DATE:	ţ	Ala Glu Tyr Pro Arg Pro Ile His Pro Ala Gly Leu Gln Pro Thr Lys 65 70 75 80
(C) CLASSIFICATION	3	Pro Leu Val Ala Thr Ser Pro Asn Pro Asp Lys Asp Gly Gly Thr Pro 85 90 95
(vii) PRIOR APPLICATION DATA: (A) APPLICATION NUMBER: 286823/1996	\$	/ Gin Giu Leu Arg Giy As
(B) FILING DATE: 29 October 1996		100 105 110
(viii) ATTORNEY/AGENT INFORMATION:	¥	n Pro Leu
(A) NAME: CONNELL, Anthony Christopher (B) GENERAL AUTHORISATION NUMBER:	\$	115 120 125
(C) REFERENCE/DOCKET NUMBER: TAK50003	Ş	Ser Ala Tyr Ala Ile Met Leu Leu Ala Leu Val Val Phe Ala Val Gly 130 135 140
(ix) 1 ELECOMMUNICATION INFORMATION: (A) TLEEPHONE: +44 127 964 4395 (B) TELEFAX: +44 181 975 6794	1	lle Val Gly Asn Leu Ser Val Met Cys lle Val Trp His Ser Tyr Tyr 145 150 155
		7 - 14 - 17 - 17 - 17 - 17 - 17 - 17 - 1

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Leu Lys Ser Ala Trp Asn Ser Ile Leu Ala Ser Leu Ala Leu Trp Asp e Val Trp His Ser Tyr Tyr 160

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175	

Phe Leu Val Leu Phe Phe Cys Leu Pro Ile Val Ile Phe Asn Glu Ile 180 185 190 Thr Lys Gln Arg Leu Leu Gly Asp Val Ser Cys Arg Ala Val Pro Phe

Thr Lys Glin Arg Leu Leu Gly Asp Val Ser Cys Arg Ala Val Pro Phe
195 200 205

Met Glu Val Ser Ser Leu Gly Val Thr Thr Phe Ser Leu Cys Ala Leu
210 215 220

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Gly lle Asp Arg Phe His Val Ala Thr Ser Thr Leu Pro Lys Val Arg 225 230 235 240 Pro lle Glu Arg Cys Gln Ser lle Leu Ala Lys Leu Ala Val lle Trp 245 250 255

Val Gly Ser Met Thr Leu Ala Val Pro Glu Leu Leu Leu Trp Gln Leu 260 265 270

Ala Gin Giu Pro Ala Pro Thr Met Gly Thr Leu Asp Ser Cys lle Met
275 280 285

Lys Pro Ser Ala Ser Leu Pro Giu Ser Leu Tyr Ser Leu Val Met Thr

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Tyr Gin Asn Ala Arg Met Trp Trp Tyr Phe Gly Cys Tyr Phe Cys Leu 305 310 315 320 Pro lle Leu Phe Thr Val Thr Cys Gin Leu Val Thr Trp Arg Val Arg

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Gly Pro Pro Gly Arg Lys Ser Glu Cys Arg Ala Ser Lys His Glu Gln 340 345 350

Cys Glu Ser Gln Leu Asn Ser Thr Val Val Gly Leu Thr Val Val Tyr 355 360 365

Ala Phe Cys Thr Leu Pro Glu Asn Val Cys Asn lle Val Val Ala Tyr 370 375 380

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Leu Ser Thr Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly Leu Ile 385 390 395 400

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Asn Gin Phe Ser Thr Phe Phe Lys Gly Ala Ile Thr Pro Val Leu Leu 405 410 415

Leu Cys Ile Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys Cys Cys 420 425 430

Cys Cys Cys Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser Ala Ala 435 440 445

Asn Gly Ser Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser Ile Tyr 450 455 460

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Phe His Lys Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly Thr Pro 465 470 475 480 Cys

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(3) INFORMATION FOR SEQ ID NO: 2

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 542

(B) TYPE: amino acid
(C) STRANDEDNESS:

(D) TOPOLOGY: linear

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(ii) MOLECULAR TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Cys Pro Ala Glu Gly Pro Ala Arg Pro Val Ala Gly Gly Trp Glu 1 5 10 15

Gly Gly Gln Ala Ser Asp Ala Arg Arg Leu Thr Gly Gly Gly Ser Ser 20 25 30

Arg Pro Ala Ala Ser Leu Glu Pro Ser Ser Trp Ala Pro Cys Thr His 35 40 45

Leu Leu Phe Leu Gly Trp Leu Ser Pro Ala His Pro Ala Met Arg Trp

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Leu Trp Pro Leu Ala Val Ser Leu Ala Val Ile Leu Ala Val Gly Leu

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Ser Arg Val Ser Gly Gly Ala Pro Leu His Leu Gly Arg His Arg Ala 85 90 95

Glu Thr Gln Glu Gln Ger Arg Ser Lys Arg Gly Thr Glu Asp Glu 100 105

Giu Aia Lys Gly Val Gin Gin Tyr Val Pro Giu Giu Trp Ala Giu Tyr 115 120

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Pro Arg Pro Ile His Pro Ala Gly Leu Gln Pro Thr Lys Pro Leu Val 130 135 Ala Thr Ser Pro Asn Pro Asp Lys Asp Gly Gly Thr Pro Asp Ser Gly
145 150 155 160
Gln Glu Leu Arg Gly Asn Leu Thr Gly Ala Pro Gly Gln Arg Leu Gln
165 170 175

lle Gln Asn Pro Leu Tyr Pro Val Thr Glu Ser Ser Tyr Ser Ala Tyr 180 185 190

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Ala IIe Met Leu Leu Ala Leu Val Val Phe Ala Val Gly IIe Val Gly 195 200 205

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Asn Leu Ser Val Met Cys lle Val Trp His Ser Tyr Tyr Leu Lys Ser 210 215 220 Ala Trp Asn Ser lle Leu Ala Ser Leu Ala Leu Trp Asp Phe Leu Val 225 230 230 235 240 Leu Phe Phe Cys Leu Pro lle Val Ile Phe Asn Glu lle Thr Lys Gln 245 250 Arg Leu Leu Gly Asp Val Ser Cys Arg Ala Val Pro Phe Met Glu Val 260 265 270

Ser Ser Leu Gly Vai Thr Thr Phe Ser Leu Cys Ala Leu Gly Ile Asp 275 280 285 Arg Phe His Val Ala Thr Ser Thr Leu Pro Lys Val Arg Pro Ile Glu 290 295

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Arg Cys Gln Ser Ile Leu Ala Lys Leu Ala Val Ile Trp Val Gly Ser

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305 310 315 320 Met Thr Leu Ala Val Pro Glu Leu Leu Leu Trp Gln Leu Ala Gln Glu 325 330 335

Pro Ala Pro Thr Met Gly Thr Leu Asp Ser Cys Ile Met Lys Pro Ser 340 345 350 Ala Ser Leu Pro Glu Ser Leu Tyr Ser Leu Val Met Thr Tyr Gln Asn 355 360 365

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Ala Arg Met Trp Trp Tyr Phe Gly Cys Tyr Phe Cys Leu Pro lle Leu 370 375 380

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 Phe Thr Val Thr Cys Gln Leu Val Thr Trp Arg Val Arg Gly Pro Pro

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 Gly Arg Lys Ser Glu Cys Arg Ala Ser Lys His Glu Gln Cys Glu Ser
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Gin Leu Asn Ser Thr Val Val Gly Leu Thr Val Val Tyr Ala Phe Cys 420 425 430 Thr Leu Pro Glu Asn Val Cys Asn Ile Val Val Ala Tyr Leu Ser Thr 435 440 445

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Giu Leu Thr Arg Gin Thr Leu Asp Leu Leu Gly Leu Ile Asn Gin Phe 450 . 455 460

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Cys Glu Glu Cys Gly Gly Ala Ser Glu Ala Ser Ala Asn Gly Ser 500 505 510

Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser Ile Tyr Phe His Lys 515 520 525

20

Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly Thr Pro Cys 530 535 540

(4) INFORMATION FOR SEQ ID NO: 3

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1443
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGCGGTGGC TGTGGCCCCT GGCTGTCTCT CTTGCTGTGA TTTTGGCTGT GGGGCTAAGC

AGGGTCTCTG GGGGTGCCCC CCTGCACCTG GGCAGGCACA GAGCCGAGAC

8

CAGAGCCGAT CCAAGAGGGG CACCGAGGAT GAGGAGGCCA
AGGGCGTGCA GCAGTATGTG

CCTGAGGAGT GGGCGGAGTA CCCCCGGCCC ATTCACCCTG CTGGCCTGCA GCCAACCAAG

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CCCTTGGTGG CCACCAGCCC TAACCCCGAC AAGGATGGGG GCACCCCAGA

GAACTGAGGG GCAATCTGAC AGGGGCACCA GGGCAGAGGC TACAGATCCA GAACCCCCTG

TATICCGGTGA CCGAGAGCTC CTACAGTGCC TATGCCATCA TGCTTCTGGC
GCTGGTGGTG

TITGCGGTGG GCATTGTGGG CAACCTGTCG GTCATGTGCA TCGTGTGGCA CAGCTACTAC

CTGAAGAGCG CCTGGAACTC CATCCTTGCC AGCCTGGCCC TCTGGGATTT
CTGGTCCTC

TTITTCTGCC TCCCTATTGT CATCTTCAAC GAGATCACCA AGCAGAGGCT
ACTGGGTGAC
600

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GITTCTIGTC GTGCCGTGCC CTTCATGGAG GTCTCCTCTC TGGGAGTCAC GACTTTCAGC

660
CTCTGTGCCC TGGGCATTGA CCGCTTCCAC GTGGCCACCA GCACCCTGCC
CAAGGTGAGG

CCCATCGAGC GGTGCCAATC CATCCTGGCC AAGTTGGCTG TCATCTGGGT GGGCTCCATG 780 ACGCTGGCTG TGCCTGAGCT CCTGCTGTGG CAGCTGGCAC AGGAGCCTGC

15
CCCCACCATG

840
GGCACCCTGG ACTCATGCAT CATGAAACCC TCAGCCAGCC TGCCCGAGTC
CCTGTATTCA

CTGGTGATGA CCTACCAGAA CGCCCGCATG TGGTGGTACT TTGGCTGCTA
CTTCTGCCTG
960

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CCCATCCTCT TCACAGTCAC CTGCCAGCTG GTGACATGGC GGGTGCGAGG CCCTCCAGGG 1020 AGGAAGTCAG AGTGGAGGGG CAGGAAGGAG GAGGAGTGTG

AGGAAGTCAG AGTGCAGGGC CAGCAAGCAC GAGCAGTGTG AGAGCCAGCT CAACAGCACC

GTGGTGGGCC TGACCGTGGT CTACGCCTTC TGCACCCTCC CAGAGAACGT
CTGCAACATC
1140

GTGGTGGCCT ACCTCTCCAC CGAGCTGACC CGCCAGACCC TGGACCTCCT
GGGCCTCATC

AACCAGTTCT CCACCTTCTT CAAGGGCGCC ATCACCCCAG TGCTGCTCCT
TTGCATCTGC
1260

AGGCCGCTGG GCCAGGCCTT CCTGGACTGC TGCTGCTGCT GCTGCTGTGA GGAGTGCGGC

GGGGCTTCGG AGGCCTCTGC TGCCAATGGG TCGGACAACA AGCTCAAGAC CGAGGTGTCC

TCTTCCATCT ACTTCCACAA GCCCAGGGAG TCACCCCCAC TCCTGCCCCT
GGGCACACCT

1440	CTGTATCCGG TGACCGAGAG CTCCTACAGT GCCTATGCCA TCATGCTTCT
1443	600 600 GIGITTIGGG TGGGCATTGT GGGCAACCTG TCGGTCATGT GCATCGTGT
(5) INFORMATION FOR SEQ ID NO: 4 (i) SEQIENCE CHAPACTEDISTICS.	GCACIGCAC ICCCOMING COCCURRED ICCCOMING CONTROLL OF THE PROPERTY OF THE PROPER
(A) LENGTH: 1626	TACCTGAAGA GCGCCTGGAA CTCCATCCTT GCCAGCCTGG CCCTCTGGGA
(B) TYPE: nucleic acid	TTTCTGGTC 720
(D) TOPOLOGY: linear 15	CTCTITITICT GCCTCCCTAT TGTCATCTTC AACGAGATCA CCAAGCAGAG
(ii) MOLECULAR TYPE: cDNA	780 Caroner presents present and caroner a
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:	GACGITICTI GICGIGCCOI GCCCITCATO GAGGICTCCI CICTUGGAGTI CACGACTTTC 840
ATGTGTCCAG CAGAGGGCCC TGCCCGGCCT GTGGCCGGAG GCTGGGAGGG AGGCAGGCG	AGCCTCTGTG CCCTGGGCAT TGACCGCTTC CACGTGGCCA CCAGCACCT
52 09	006
AGTGATGCCA GACGCCTGAC TGGAGGCGGA TCCAGCCGGC CAGCTGCCTC TCTGGAGCCC	AGGCCCATCG AGCGGTGCCA ATCCATCCTG GCCAAGTTGG CTGTCATCTG GGTGGGCTCC
120 AGCTCTTGGG CCCCCTGTAC TCACCTGCTC TTCCTGGGCT GGCTGTCTCC	960 ATGACGCTGG CTGTGCCTGA GCTCCTGCTG TGGCAGCTGG CACAGGAGCC
TGCTCATCCA 180	TGCCCCACC 1020
GCCATGCGGT GGCTGTGGCC CCTGGCTGTC TCTCTTGCTG TGATTTTGGC TGTGGGGCTA	ATGGGCACCC TGGACTCATG CATCATGAAA CCCTCAGCCA GCCTGCCCGA GTCCCTGTAT
490 AGCAGGGTCT CTGGGGGTGC CCCCCTGCAC CTGGGCAGGC ACAGAGCCGA GACCCAGGAG	1080 TCACTGGTGA TGACCTACCA GAACGCCCGC ATGTGGTGGT ACTTTGGCTG CTACTTCTGC
SUS CAGCAGAGCC GATCCAAGAG GGGCACCGAG GATGAGGAGG	CTGCCCATCC TCTTCACAGT CACCTGCCAG CTGGTGACAT GGCGGGTGCG
CCAAGGGCGT GCAGCAGTAT	AGGCCTCCA 1200
GTGCCTGAGG AGTGGGCGGA GTACCCCCGG CCCATTCACC CTGCTGGCCT GCAGCCAACC	GGGAGGAAGT CAGAGTGCAG GGCCAGCAAG CACGAGCAGT GTGAGAGCCA GCTCAACAGC
420	1260
AAGCCCTTGG TGGCCACCAG CCCTAACCCC GACAAGGATG GGGGCACCCC	ACCGTGGTGG GCCTGACCGT GGTCTACGCC TTCTGCACCC TCCCAGAGAA
480	1320
540 CAGGAACTGA GGGGCAATCT GACAGGGGCA CCAGGGCAGA GGCTACAGAT CCAGAACCCC	

ATCAACCAGT TCTCCACCTT CTTCAAGGGC GCCATCACCC CAGTGCTGCT ATCGTGGTGG CCTACCTCTC CACCGAGCTG ACCCGCCAGA CCCTGGACCT CCTGGGCCTC

CCTTTGCATC

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TGCAGGCCGC TGGGCCAGGC CTTCCTGGAC TGCTGCTGCT GCTGCTGCTG TGAGGAGTGC

GACCGAGGTG GGCGGGGCTT CGGAGGCCTC TGCTGCCAAT GGGTCGGACA ACAAGCTCAA

TCCTCTTCCA TCTACTTCCA CAAGCCCAGG GAGTCACCCC CACTCCTGCC

CCTGGGCACA

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CCTTGC

(6) INFORMATION FOR SEQ ID NO: 5

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 426

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTGGCTGTGC TGCAATCCAT CCTGGCCAAG TTGGCTGTCA TCTGGGTGGG CTCCATGACG

ŝ ACCCTGGACT CTGAGCTCCT GCTGTGGCAG CTGGCACAGG AGCCTGCCCC CACCATGGGC

CATGCATCAT GAAACCCTCA GCCAGCCTGC CCGAGTCCCT GTATTCACTG GTGATGACCT

60

ACCAGAACGC CCGCATGTGG TGGTACTTTG GCTGCTACTT CTGCCTGCCC ATCCTCTTCA

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CAGTCACCTG CCAGCTGGTG ACATGGCGGG TGCGAGGCCC TCCAGGGAGG AAGTCAGAGT

30

GTGGGCCTGA CCGTGGTCTA CGGCTTTTTG CAACCTTCCA GAGAACGTTT GCAACATCGT GCAGGGCCAG CAAGCACGAG CAGTGTGAGA GCCAGCTCAA CAGCACCGTG

GGTGGGCTTA

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CCTTTT 426

(7) INFORMATION FOR SEQ ID NO: 6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 248

8

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

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(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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ACATCGTGGT AACAAGGGCC GTGGTCTACG NCTTCTGCAC CCTCCCANAG AACGTCTGCA

GGCCTACCTC TCCACCGAGC TGACCCGCCA GNCCCTGGAC CTCCTGGGCC TCATCAACCA

GTTCTCCACC TTCTTCAAGG GCGCCATCAC CCCAGTGCTG CTCCTTTGCA TCTGCAGGCC

GCGGCGGGC GCTGGGCCAG GCCTTCCTGG ACTGCTGCTG CTGCTGCTGC TGTNAGGAGT

TTCGGAGG

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(8) INFORMATION FOR SEQ ID NO: 7

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

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(ii) MOLECULAR TYPE: synthetic DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10: GAAGAGGATG GGCAGGCAGA AGTAGCAG 28	(12) INFORMATION FOR SEQ ID NO: 11 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 (B) TYPE: micleic poid	(c) Tree, moters and (c) STRANDEDNESS: single (D) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:	ATGAAGGGCA CGGCACGACA AGAAACG 27	(13) INFORMATION FOR SEQ ID NO: 12 (1) SEOI FINCE CHARACTERISTICS:	(A) LENGTH: 27 (B) TYPE: nucleic acid (C) STRANDEDNESS: single	(ii) MOLECULAR TYPE: synthetic DNA	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:	ATGACAATAG GGAGGCAGAA AAAGAGG 27	(14) INFORMATION FOR SEQ ID NO: 13 (1) SEQUENCE CHARACTERISTICS: (A) I ENGTH: 33	(b) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA
Va	01	31	20	52		30	35	40		\$ 1	09	55
(B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULAR TYPE: synthetic DNA	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7: AAGTTGGCTG TCATCTGGGT GGGCTC 26 (9) NFORMATION FOR SEO ID NO: 8	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 28 (B) TYPE: Intelest acid	(C) S1 KANDEDNESS: single (D) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA	(x) Sectional Description: Section (x) Sectional Transfer of Trans	(10) INFORMATION FOR SEQ ID NO: 9 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 (B) TYPE: michaic acid	(C) STRANDEDNESS: single (D) TOPOLOGY: linear	(ii) MOLECULAR TYPE: synthetic DNA	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:	CATGCGGGCG TTCTGGTAGG TCATCAC 27	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 28 (B) TYPE: nucleic acid (C) STRANDEDNESS: single	(D) IOPOLOGY: Innear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13: GTCGACGAGA TGTGTGAGGG CAGCAAAGAG TGC ¥

(i) SEQUENCE CHARACTERISTICS: (15) INFORMATION FOR SEQ ID NO: 14

(A) LENGTH: 28

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: synthetic DNA

TACTGGGGCC TCAGCAAGGT GTGCCCAG 28 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

8

(16) INFORMATION FOR SEQ ID NO: 15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32

23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(ii) MOLECULAR TYPE: synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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GTCGACTGGC TGTCTCCTGC TCATCCAGCC AT 32

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SEQUENCE LISTING

INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 481
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

7

(ii) MOLECULAR TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Glu Asp Glu Glu Ala Lys Gly Val Gln Gln Tyr Val Pro Glu Glu Trp His Arg Ala Glu Thr Gin Giu Gin Gin Ser Arg Ser Lys Arg Gly Thr Val Gly Leu Ser Arg Val Ser Gly Gly Ala Pro Leu His Leu Gly Arg Met Arg Trp Leu Trp Pro Leu Ala Val Ser Leu Ala Val Ile Leu Ala Asp Ser Gly Gln Glu Leu Arg Gly Asn Leu Thr Gly Ala Pro Gly Gln Pro Leu Val Ala Thr Ser Pro Asn Pro Asp Lys Asp Gly Gly Thr Pro Ala Glu Tyr Pro Arg Pro Ile His Pro Ala Gly Leu Gln Pro Thr Lys 65 8 35 00 T 8 7 SS 6 105 8 75 8 10 95 15 80

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lle, Gln Asn Pro Leu Tyr Pro Val Thr	115 120 125	Ser Ala Tyr Ala Ile Met Leu Leu Ala Leu Val Val Phe Ala Val Gly	130 135 140	Ile Val Gly Asn Leu Ser Val Met Cys Ile Val Trp His Ser Tyr Tyr	145 150 155 160	Leu Lys Ser Ala Trp Asa Ser Ile Leu Ala Ser Leu Ala Leu Trp Asp	165 170 175	Phe Leu Val Leu Phe Phe Cys Leu Pro Ile Val Ile Phe Asn Glu Ile	180 185 190	Thr Lys Cin Arg Leu Leu Gly Asp Val Ser Cys Arg Ala Val Pro Phe	195 . 200 205	Met Glu Val Ser Ser Leu Gly Val Thr Thr Phe Ser Leu Cys Ala Leu	210 215 220	Gly lle Asp Arg Phe His Val Ala Thr Ser Thr Leu Pro Lys Val Arg	225 · 230 235 240	Pro Ile Glu Arg Cys Gln Ser Ile Leu Ala Lys Leu Ala Val Ile Trp	245 250 255	Val Gly Ser Met Thr Leu Ala Val Pro Glu Leu Leu Leu Trp Gln Leu	260 265 270	Ala Gin Giu Pro Ala Pro Thr Met Giy Thr Leu Asp Ser Cys Ile Met	275 280 . 285

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Thr		Leu	320	Arg		Glb		Tyr		Tyr		11e	400	Leu		Cys		Ala		Iyr		Pro	480	
			60										4		LO.								4	
Het		Cys		Val	335	Glu		Val		Ala		Leu		Leu	415	Çys		Ala		116		Thr		
Val		Pbe		ÅrB		H:s	350	Val		Val		G1,y		Val		Cys	430	Ser		Ser		Gly		
Leu		Iyr		Irp		Lys		Ţļ.	365	Val		Leu		Pro		Asp		Ala	445	Ser		Leu		
Ser	300	Cys		Thr		Ser		Leu		11e	380	Leu		Į.		Leu		ចូ		Ser	460	Pro		
Tyr		Gly	315	Val		Ala		G13		Asn		Asp	395	116		Phe		Ser	-	Val		Leu	475	
Leu		Phe		Leu	330	Arg		Val		Cys		Leu		Ala	410	A18		Ala		Glu		Leu		
Ser		Iyr		Gla		Cys	345	Val		Val		Thr		Gly		Gla	425	61,		Thr		Pro		
G1 _u		Irp		Cys		G] u		Thr	360	Asn		Gla		Lys		G1,		Gly	440	Lys		Pro		
Pro	295	Ĭŗ		Thr		Ser		Ser		n [5	375	Arg		Phe		Leu		Cys		Leu	455	Ser		
Leu		Met	310	Val		Lys		Asn		Pro		Ibr	390	Phe		Pro		G1.		Lys		Glu	470	
Ser		Årß		Thr	325	Arg		Leu		Leu		Leu		Th.	405	Arg		Glu		Asn		Arg		*
Ala		Ala		Phe		G13	340	Gln		Thr		67.4		Ser		Cys	420	Ç		Asp		Pro		
Ser		Asn		Leu		Pro		Ser	355	Çys		Ţ		Phe		116		Cys	435	Ser		Lys		
Pro	290	619		11e		Pro		GIu		Phe	370	Ser		G12		Cys		Cys		613	420	H.		
Lys		Iyr	305	Pro		Gly		Cys		Ala		Leu	385	Asn		Len		Cys		Asa		Phe	465	Cys

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SEQUENCE CHARACTERISTICS: A) LENGTH: 542 B) TYPE: anino accid C) STRANDEDNESS: D) TOPOLOGY: linear) MOLECULAR TYPE: peptide) MOLECULAR TYPE: peptide Cys Pro Ala Giu Gly Pro Ala Arg Pro Val Ala Gly Gly Trp Glu Cys Pro Ala Giu Gly Pro Ala Arg Pro Val Ala Gly Gly Trp Glu Cys Pro Ala Ser Asp Ala Arg Arg Leu Thr Gly Gly Gly Ser Ser Gly Gln Ala Ser Leu Glu Pro Ser Ser Trp Ala Pro Cys Thr His 20 25 35 40 45 Leu Phe Leu Gly Trp Leu Ser Pro Ala His Pro Ala Met Arg Trp 50 55 56 Fro Ala Ala Ser Gly Gly Ala Pro Leu His Leu Ala Val Gly Leu Trp Pro Leu Ala Val Ser Leu Ala Val Ile Leu Ala Val Gly Leu Trp Pro Glu Glu Gln Ser Arg Ser Lys Arg Gly Thr Glu Asp Glu 100 100 105 116 117 118 120 129 135 140 150 150 150 150 150	145	λla		Pro		G1u		01u		Ser	65	Leu		Leu		8.1		¥ Li	-	et -	(xi)	(ii)	S (4)
ICE CHARACTERISTICS: "TH: 542 "I amino acid NDEDNESS: LOGY: linear CULAR TYPE: peptide ENCE DESCRIPTION: SEQ ID NO: 2: Ala Giu Gly Pro Ala Arg Pro Val Ala Gly Gly Trp (5 10 15 Ala Ser Asp Ala Arg Arg Leu Thr Gly Gly Gly Ser 20 25 30 Ala Ser Leu Glu Pro Ser Ser Trp Ala Pro Cys Thr 40 40 45 Leu Gly Trp Leu Ser Pro Ala His Pro Ala Met Arg 60 Leu Ala Vel Ser Leu Ala Val Ile Leu Ala Val Gly 70 Ser Gly Gly Ala Pro Leu His Leu Gly Arg His Arg 61 Glu Gln Gln Ser Arg Ser Lys Arg Gly Thr Glu Asp 100 Gly Val Gln Gln Tyr Val Pro Glu Glu Trp Ala Glu 120 110 120 135 He His Pro Ala Gly Leu Gln Pro Thr Lys Pro Leu 135 140 Pro Asn Pro Asp Lys Asp Gly Gly Thr Pro Asp Ser 150		The	130	λrg		λla		Th		λrg		Trp	8					51y (TS TEC
CE CHARACTERISTICS: ### 1542 Camino acid MDEDNESS: LOGY: linear LOGY: linear				Pro	115	Lys		G1 _b		Val		Pro			ဒ္ဌ			Cin.		Pro /	E O)LEC	YPE YPE OPO
HARACTERISTICS: \$150.0 acid NESS: \$1 linear TYPE: peptide DESCRIPTION: SEQ ID NO: 2: Lu Gly Pro Ala Arg Pro Val Ala Gly Gly Trp (\$10						GLy	100	G1u		Ser		Leu		īeī.			20			Ala a	NCE	g	TH:
ERRISTICS: PERISTICS: PAR PAR PERISTICN: SEQ ID NO: 2: Pro Ala Arg Pro Val Ala Gly Gly Trp (10 15 Ala Arg Arg Leu Thr Gly Gly Gly Trp (25 30 25 30 Glu Pro Ser Ser Trp Ala Pro Cys Thr 40 45 Leu Ser Pro Ala His Pro Ala Met Arg 55 80 Ser Leu Ala Val Ile Leu Ala Val Gly 75 Ala Pro Leu His Leu Gly Arg His Arg 90 95 Ser Arg Ser Lys Arg Gly Thr Glu Asp 120 125 Ala Gly Leu Gln Pro Thr Lys Pro Leu 135 140 Asp Lys Asp Gly Gly Thr Pro Asp Ser 155						Val		uT.	85	G1.y		λla		Gly					ຜ	01 n		7	54X 54X ninc DNES
ptide ON: SEQ ID NO: 2: Ala Arg Pro Val Ala Gly Gly Trp (10 15 Arg Arg Leu Thr Gly Gly Gly Ser 25 30 25 30 Pro Ser Ser Trp Ala Pro Cys Thr 40 45 Ser Pro Ala His Pro Ala Met Arg 60 Leu Ala Val Ile Leu Ala Val Gly 75 Pro Leu His Leu Gly Arg His Arg 90 95 Arg Ser Lys Arg Gly Thr Glu Asp 105 110 120 125 Gly Leu Gln Pro Thr Lys Pro Leu 140 Lys Asp Gly Gly Thr Pro Asp Ser 155	150					C1 n		g 15		G1.y	70	Va1		Trp		Leu		λsp			Č	. 3 <i>q</i> 7	ACT SS:
STICS: Description Description Description			135			G1 ⁿ				Ala		202	ទ	Leu				λla		Pro	TTG		id
EQ ID NO: 2: Pro Val Ala Gly Gly Trp (10 15 10 15 10 15 Reu Thr Gly Gly Gly Ser 30 60 Ala His Pro Ala Met Arg 80 90 11 Pro Glu Gly Thr Glu Asp 125 110 1 Pro Glu Glu Trp Ala Glu 125 140 140 155					120	Tyr						Leu			40			λrg		Ala	8 8	pti	STIC
O: 2: Ala Gly Gly Trp (Ala Gly Gly Ser Gly Gly Gly Ser Ala Pro Cys Thr 45 Pro Ala Met Arg 60 Gly Arg His Arg 60 Gly Arg His Arg 110 Glu Trp Ala Glu 125 Gly Thr Lys Pro Leu 140 Thr Lys Pro Asp Ser		λsp					105	Ser		Leu		Ala		Pro			25	٨гв			SH	ė	53.
O: 2: Ala Gly Gly Trp (Ala Gly Gly Ser Gly Gly Gly Ser Ala Pro Cys Thr 45 Pro Ala Met Arg 60 Gly Arg His Arg 60 Gly Arg His Arg 110 Glu Trp Ala Glu 125 Gly Thr Lys Pro Leu 140 Thr Lys Pro Asp Ser		Gly		C1n	•				90	His		Val		Ala		Ser		Leu	10				
O: 2: Ala Gly Gly Trp (Ala Gly Gly Ser Gly Gly Gly Ser Ala Pro Cys Thr 45 Pro Ala Met Arg 60 Gly Arg His Arg 60 Gly Arg His Arg 110 Glu Trp Ala Glu 125 Gly Thr Lys Pro Leu 140 Thr Lys Pro Asp Ser	155	£19								Leu	75	110		His		Trp				Val			
Gly Gly Trp (Gly Gly Ser 15 Gly Gly Ser 30 Pro Cys Thr 45 Ala Met Arg Ala Val Gly Ala Val Gly Ilo Irp Ala Glu 125 Fro Asp Ser	•		140			ត្		Gly		G1 y			89	Pro		λla		GLy		Ala			
Gly Trp (15 15 Gly Ser Gly Ser 7 Glu Asp Pro Leu Pro Leu Asp Ser Asp Ser			_		125	Trp		Ħ		Arg		λla		λla	45	Pro		G1 y		Gly	:-		
							110	113		#is		Val					ಜ	G1y					
						G1 _u		Asp	95			Gly		Arg					15	Trp			
	160			Val				113		Ala	8	Leu		Trp		His		Ser		Glu			
	-	-																					
																					-		

355	Ala Ser Leu Pro Glu	340	Pro Ala Pro Thr Met	325	Met Thr Leu Ala Val	305	Arg Cys.Gln Ser Ile	290	Arg Phe His Val Ala	275	Ser Ser Leu Gly Val	. 260	Arg Leu Leu Gly Asp	245	Leu Phe Phe Cys Leu	225	Ala Trp Asn Ser Ile l	210	Asn Leu Ser Val Met (195	Ala Ile Met Leu Leu Ala	. 180	Ile Gln Asn Pro Leu T	165	Gln Glu Leu Arg Gly Ai
360	Ser Leu Tyr Ser Leu Val	345	Gly Thr Leu Asp Ser Cys	330	Pro Glu Leu Leu Irp	310 315	Leu Ala Lys Leu Ala Val	295	Thr Ser Thr Leu Pro Lys	280	Thr Thr Phe Ser Leu Cys	265	Val Ser Cys Arg Ala Val	250	Pro Ile Val Ile Phe Asn	230 235	Leu Ala Ser Leu Ala Leu	215	Cys Ile Val Trp His Ser	200	la Leu Val Val Phe Ala	185	Tyr Pro Val Thr Glu Ser	170	Asn Leu Thr Gly Ala Pro
365	al Met Thr Tyr Gln Asn	350	s Ile Met Lys Pro Ser	335	p Gln Leu Ala Gln Glu	5 320	l lle Trp Val Gly Ser	300	s Val Arg Pro Ile Glu	285	s Ala Leu Gly Ile Asp	270	Pro Phe	255	Glu Ile Thr Lys Gln	240	Irp Asp Phe Leu Val	220	Tyr Tyr Leu Lys Ser	205	Val Gly Ile Val Gly	190	Ser Tyr Ser Ala Tyr	175	Gly Gln Arg Leu Gln

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	45	530 535 540
		Pro Arg Glu Ser Pro Pro Leu Leu Pro Leu Gly Thr Pro Cys
		515 520 525
	40	Asp Asn Lys Leu Lys Thr Glu Val Ser Ser Ser Ile Tyr Phe His Lys
		500 505 510
	35	Cys Glu Glu Cya Gly Gly Ala Ser Glu Ala Ser Ala Ala Asn Gly Ser
TITGCGTGG GCATTGTGGG CAACCT		485 490 495
TATCCGTGA CCGAGAGCTC CTACAG		Cys Arg Pro Leu Gly Gln Ala Phe Leu Asp Cys Cys Cys Cys Cys
GAACTGAGGG GCAATCTGAC AGGGGC	30	465 470 475 480
CCCTTGGTGG CCACCAGCCC TAACCC		Ser Thr. Phe Phe Lys Gly Ala Ile Thr Pro Val Leu Leu Leu Cys Ile
CCTGAGGAGT GGGCGGAGTA CCCCCG	25	450 455 460
CAGAGCCGAT CCAAGAGGGG CACCGA		Glu Leu Thr Arg Gln Thr Leu Asp Leu Leu Gly Leu Ile Asn Gln Phe
AGGGTCTCTG GGGGTGCCCC CCTGCA	ì	435 440 445
ATGCGGTGGC TGTGGCCCCT GCCTGT	66	Thr Leu Pro Glu Asn Vel Cys Asn Ile Vel Vel Ale Tyr Leu Ser Thr
		420 425 430
	15	Gin Leu Asn Ser Thr Val Val Gly Leu Thr Val Val Tyr Ala Phe Cys
(xi) SEQUENCE DESCRIPT		405 410 415
(ii) MOLECULAR TYPE:	10	Gly Arg Lys Ser Glu Cys Arg Ala Ser Lys His Glu Gln Cys Glu Ser
		385 390 395 400
(B) TYPE: nucleic ac (C) STRANDEDNESS: dc		Phe Thr Val Thr Cys Gln Leu Val Thr Trp Arg Val Arg Gly Pro Pro
(i) SEQUENCE CHARACTEI (A) LENGTH: 1443	s	370 375 380
information for seq ii		Ala Arg Met Trp Trr Phe Gly Cys Tyr Phe Cys Leu Pro Ile Leu

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he Cys Leu Pro Ile Leu	INFORMATION FOR SEQ ID NO: 3	
380	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1443	
rg Val Arg Gly Pro Pro	(B) TYPE: nucleic acid (C) STRANDEDNESS: double	
95 400		
is Glu Gla Cys Glu Ser	" (ii) MOLECULAR TYPE: cDNA	
415	(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 3:	
al Val Iyr Ala Phe Cys	15	
430		
al, Ala Tyr Leu Ser Thr	ATGCGGIGGC IGIGGCCCCI GGCIGICICI CIIGCTGIGA IIIIGGCIGI GGGGCIAAGC	
.445	20 AGGGICICIG GGGGIGCCCC CCIGCACCIG GGCAGGCACA GAGCCGAGAC CCAGGAGCAG	
Cly Leu Ile Asn Gln Phe	CAGAGCGGAI CCAAGAGGGG CACCGAGGAI GAGGAGGCCA AGGGCGIGCA GCAGIAIGIG	
460	25 CCIGAGGAGI GGGCGGAGIA CCCCGGCCC AITCACCCIG CIGGCCIGCA GCCAAG	
Val Leu Leu Leu Cys 11e	CCCTIGGIGG CCACCAGCC TAACCCCGAC AAGGAIGGGG GCACCCCAGA CAGIGGGCAG	
475 480	GAACTGAGGG GCAATGTGAG AGGGGACCA GGGCAGAGGC TACAGATCCA GAACGCGTG	(7
Cys Cys Cys Cys Cys	TATCCGGTGA CCGAGAGCTC CTACAGTGCC TATGCCATCA TGCTTCTGGC GCTGGTGGTG	17
495	TIIGCGGIGG GCAIIGIGGG CAACCIGICG GICAIGIGCA ICGIGIGGCA CAGCIACIAC	E.S
Ser Ala Ala Asn Gly Ser	35	
510		
Ser Ile Tyr Phe His Lys	07	
525		
Gly Thr Pro Cys		
540	99	

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GGGGCTTCGG	GGGGCTTCC	•	AGGCCGCTG	AACCAGITCI	GIGGIGGCC	CIGGIGGG	AGGAAGICA	CCCATCCTCT	CIGGIGAIG	GCCACCCIG	ACCCTGCCT	CCCATCGAG	CICIGIGCC	GIIICIIGI	TTTTTCTGC	CTGAAGAGC
	TOTICOATOT ACTICCACAA GCCCAGGGAG TCACCCCAC TCCTGCCCCI GGGCACACCI	3G AGGCCICIGC IGCCAAIGGG ICGGACAACA AGCICAAGAC CGAGGIGICC	AGGCCGCIGG GCCAGGCCII CCIGGACIGC IGCIGCIGCI GCIGCIGIGA GGAGIGCGGC	I CCACCITCII CAAGGGCGCC ATCACCCCAG IGCIGCICCI TIGCAICIGC	GIGGIGGCCI ACCIGICCAC CGAGCIGACC CGCCAGACCC IGGACCICCI GGGCCICAIC	GIGGIGGGCC IGACCGIGGI CIACGCCIIC IGCACCCICC CAGAGAACGI CIGCAACAIC	AGGAAGTCAG AGTGCAGGGC CAGCAAGCAC GAGCAGTGTG AGAGCCAGCT CAACAGCACC	T TEACAGTEAC CIGECAGEIG GIGACATGGE GEGIGEGAGG CECTECAGGG	CIGGIGAIGA CCIACCAGAA CGCCCCCAIG IGGIGGIACI IIGGCIGCIA CIICIGCCIG	GGCACCCIGG ACTCAIGCAI CAIGAAACCC ICAGCCAGCC IGCCCGAGIC CCIGIAIICA	ACCCTGGCTG TGCCTGAGCT CCTGCTGTGG CAGCTGGCAC AGGAGCCTGC CCCCACCATG	CCCATCGAGC GGTGCCAATC CATCCTGGCC AAGITGGCTG ICATCTGGGT GGGGTCCATG	CICIGIGCCC IGGGCAIIGA CCGCIICCAC GIGGCCACCA GCACCCIGCC CAAGGIGAGG	GITICITGIC GIGCCGIGCC CITCAIGGAG GICICCICIC IGGGAGICAC GACITICAGC	TITITCTGCC TCCCTATTGT CATCTICAAC GAGATCACCA AGCAGAGGCT ACTGGGTGAC	CIGAAGAGCG CCIGGAACIC CAICCIIGCC AGCCIGGCCC ICIGGGAIII ICIGGICCIC
1440	CACCT 1440	TGTCC 1380	GCGGC 1320	TCTGC 1260	TCATC 1200	NCATC 1140	3CACC 1080	CAGGG 1020	CCTG 960	VIICA 900	CATG 840	CATG 780	GAGG 720	.CAGC 660	TGAC 600	CCIC 540
		٤	3		25		20			15		10			U n	
					GCCATGCGGT GGCTGTGGCC CCTGGCTGTC TCTCTTGCTG TGATTTTGGC TGTGGGGCCTA 240	AGCTETTEGG CCCCCTGTAC TCACCTGCTC TTCCTGGGCT GGCTGTCTCC TGCTCATCCA	AGTGATGCCA GACGCCTGAC IGGAGGGGGA TCCAGGCGGG CAGCTGCCTC ICTGGAGGCC	ATGTGTCCAG CAGAGGGCC TGCCCGGCCT GTGGCCGAG CCTGGGAGGG AGGGCAGGCG			(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:	(ii) MOLECULAR TYPE: cDNA	(C) STRANDEDNESS: GOUDIE (D) TOPOLOGY: linear	(A) LENGTH: 1526 (B) TYPE: nucleic acid	(i) SEQUENCE CHARACTERISTICS:	INFORMATION FOR SEQ ID NO: 4

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CCAGGGTCT	CICCCCCICC	CIGGGGTGC CCCCTGCAC CTGGGCAGGC ACAGAGCCGA GACCCAGGAG	TGGGCAGGC	ACAGAGCCGA	GACCCAGGAG	300
SAGCAGAGCC	GATCCAAGAG	GATCCAAGAG GGGCACCGAG GATGAGGAGG CCAAGGGCGT	GATGAGGAGG		GCAGCAGTAT	360
TGCCTGAGG	AGTGGGCGGA	AGTGGGGGA GTACCCCGG CCCATTCACC		CIGCIGGCCI GCAGCCAACC	CAGCCAACC	420
AGCCCTTGG	TGGCCACCAG	AGCCCTTGG TGGCCACCAG CCCTAACCCC GACAAGGATG	GACAAGGATG	GGGCACCC AGACAGTGGG	AGACAGTGGG	480
CAGGAACTGA	GGGGCAATCT	AGGAACTGA GGGGCAATCT GACAGGGGCA CCAGGGCAGA GGCTACAGAT	CCAGGGCAGA	GCCIACAGAT	CCAGAACCCC	540
CTGTATCCGG	TGACCGAGAG	CIGTATCCGG IGACCGAGAG CICCTACAGI GCCTATGCCA	GCCTATGCCA	TCATGCTTCT	GCCCTGGTG	900
GTGTTTGCGG	STGITIGGG IGGGCATIGI		GGGCAACCTG TCGGTCATGT	GCAICGIGIG	GCACAGCTAC	099
TACCTGAAGA	TACCTGAAGA GCGCCTGGAA	CICCATCCII	CICCAICCII GCCAGCCIGG CCCICIGGGA	CCCTCTGGGA	TTTTCTGGTC	720
CTCTTTTCT	GCCTCCCTAT	TGTCATCTTC	TGTCATCTTC AACGAGATCA CCAAGCAGAG	CCAAGCAGAG	GCTACTGGGT	. 780
GACGITICIT	GTCGIGCCGI	GCCCTTCATG	GCCCTTCATG GAGGICTCCT	CICIGGGAGI	CACGACITIC	840
AGCCICIGIG	CCCTGGGCAT	AGCETCIGIG CCCIGGGCAI IGACGGCIIC CACGIGGCCA CCAGCACCCI	CACGTGGCCA	CCAGCACCCT	GCCCAAGGTG	900
AGGCCCATCG	AGCCCTGCCA	AGGCCCATCG AGGGTGCCA ATCCATCCTG GCCAAGTTGG CTGTCATCTG	GCCAAGTTGG	CTGTCATCTG	cerecectec	960
ATGACGCTGG		CTGTGCCTGA GCTCCTGCTG	TGGCAGCTGG	CACAGGAGCC	TGGCAGCIGG CACAGGAGCC TGCCCCCACC	1020
ATGGGCACCC	: TGGACTCATG	IGGACICAIG CAICAIGAAA CCCICAGCCA GCCIGCCCGA GICCCIGIAI	CCCTCAGCCA	GCCTGCCCGA	GICCCIGIAI	1080
TCACTGGTGA		IGACCIACCA GAACGCCCGC AIGIGGIGGI ACITIGGCIG	ATGTGGTGGT	ACTITGGCTG	CIACITCICC	1140
CTGCCCATCC	: FCTTCACAGI	CTGCCCAȚCC FCTICACAGI CACCIGCCAG CIGGIGACAI GGCGGIGGG AGGCCTICCA	CIGGIGACAI	GGGGGGTGCG	AGGCCTCCA	1200
GGGAGGAAGI	r CAGAGTGCAC	GGGAGGAAGT CAGAGTGCAG GGCCAGCAAG CACGAGCAGT	CACGAGCAGT	GTGAGAGCCA	GIGAGAGCCA GCICAACAGC	1260
ACCCIGGIGG	GCCTGACCG1	r getetacec	TICIGCACCC	TCCCAGAGAA	GCCIGACCGI GGICIACGCC IICIGCACCC ICCCAGAGAA CGICIGCAAC	1320
ATCGTGGTGG	CCTACCTCT	CACCGAGCTG	ACCCCCCAGA	CCCTGGACCT	CCTACCICIC CACCGAGCIG ACCGGCCAGA CCCIGGACCI CCIGGGCCIC	1380
ATCAACCAG	r rerecaeer	ATCAACCAGT ICTCCACCII CIICAAGGGC GCCAICACCC	GCCATCACC	CAGTGCTGCT	CCTTTGCATC	1440
TGCAGGCGG		TGGGCCAGGC CTTCCTGGAC TGCTGCTGCT	recrecreci	. פכוככופכופ	TGAGGAGTGC	1500
GGCGGGGCII	r cccacccrc	C TGCTGCCAA1	GGGTCGGACA	ACAAGCTCA!	TGCTGCCAAT GGGTCGGACA ACAAGCTCAA GACCGAGGTG	1560
TCCTCTTCC	A TCTACTICC	A CAAGCCCAGG	GAGTCACCC	: CACTCCTGC	ICCICIICCA ICIACIICCA CAAGCCCAGG GAGICACCCC CACICCIGCC COIGGGCACA	1620
CCTIGC						1628

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ATCCAAGAG GGGCACCGAG GATGAGGAGG CCAAGGGCGT GCAGCAGTAT	360
GIGGEGGA GIACCECEGG CECAITCACE CIGCIGGECI GEAGGEAACE	420
GGCCACCAG CCCTAACCCC GACAAGGAIG GGGGCACCCC AGACAGIGGG	480
GGGGAATCT GACAGGGGCA CCAGGGCAGA GGCTACAGAT CCAGAACCCC	540
GACCGAGAG CICCIACAGI GCCIAIGCCA ICAIGCIICI GGCGCIGGIG	009
GGGCATIGI GGGCAACCIG ICGGICAIGI GCAICGIGIG GCACAGCIAC	660
GGCCIGGAA CICCAICCII GCCAGCCIGG CCCICIGGGA IIIICIGGIC	720
SCCICCIAI IGICAICIIC AACGAGAICA CCAAGCAGAG GCIACIGGOI	
STEGTGGGGT GCGCTTCATG GAGGTCTCCT CTCTGGGAGT CACGACTTTC	840 20 CI
CCCIGGGCAI IGACCGCIIC CACGIGGCCA CCAGCACCCI GCCCAAGGIG	006
AGGGGTGCCA ATCCATCCTG GCCAAGTTGG CTGTCATCTG GGTGGGCTCC	096 sz
CTGTGCCTGA GCTCCTGCTG TGGCAGCTGG CACAGGAGGC TGCCCCCACC	1020
IGGACICAIG CAICAIGAAA CCCICAGCCA GCCIGCCGA GICCCIGIAI	
TGACCTACCA GAACGCCCGC ATGTGGTGGT ACTITGGCTG CTACTTCTGC	1140 30 CC
TCTICACAGI CACCIGCCAG CIGGIGACAI GGCGGGIGCG AGGCCCICCA	1200
CAGAGTGCAG GGCCAGCAAG CACGAGCAGT GTGAGAGCCA GCTCAACAGC	1260 35
GCCIGACCGI GGICIACGCC IICIGCACCC ICCCAGAGAA CGICIGCAAC	1320
CCTACCICIC CACCGAGCIG ACCCGCCAGA CCCIGGACCI CCIGGGCCIC	1380
ICICCACCII CIICAAGGGC GCCAICACCC CAGIGCIGCI CCIIIGCAIC	1440
IGGCCAGGC CTICCTGGAC IGCTGCTGCT GCTGCTGCTG TGAGGAGTGC	1500
CGGAGGCCIC IGCIGCCAAI GGGICGGACA ACAAGCICAA GACCGAGGIG	1560 45
TCIACITCCA CAAGCCCAGG GAGTCACCCC CACTCCTGCC CCTGGGCACA	1620
	1626 50

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(B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: CDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGCAATCCAT	IGGAAICCAI CCIGGCCAAG IIGGCIGICA ICIGGGIGGG CICCAIGACG CIGGCIGIGC	TIGGCIGICA	TCTGGGTGGG	CTCCATGACG	CIGGCIGIGC	09
CIGAGCICCI	CIGAGCICCI GCIGIGGCAG CIGGCACAGG AGCCIGCCC CACCAIGGGC ACCCIGGACI	CTGGCACAGG	AGCCIGCCC	CACCATGGGC	ACCCIGGACT	120
CATGCATCAT	CAIGCAICAI GAAACCCICA GCCAGCCIGC CGGAGICCCI GIAIICACIG GIGAIGACCI	GCCAGCCIGC	CCGAGTCCCT	GTATICACIG	GTGATGACCT	180
ACCAGAACGC	ACCAGAACGC CCGCAIGIGG IGGIACIIIG GCIGCIACII CIGCCIGCCC AICCICIICA	TGGTACTTTG	GCTGCTACTT	CIGCCIGCCC	ATCCTCTTCA	240
CAGTCACCTG	CAGTCACCTG CCAGCTGGTG ACAIGGCGGG IGCGAGGCCC ICCAGGGAGG AAGICAGAGI	ACATGGCGG	TGCGAGGCCC	TCCAGGGAGG	AAGTCAGAGT	300
GCAGGGCCAG	GCAGGGCCAG CAAGCACGAG CAGTGTGAGA GCCAGCTCAA CAGCACCGTG GTGGGCCTGA	CAGTGTGAGA	GCCAGCTCAA	CAGCACCGTG	GIGGGCCIGA	360
CCGTGGTCTA	CCGTGGICTA CGGCITITIG CAACCTICCA GAGAACGIII GCAACAICGI GGIGGGCITA	CAACCTTCCA	GAGAACGITI	GCAACATCGT	GGTGGGCTTA	420
CCTTT						426

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INFORMATION FOR SEQ ID NO: 6

INFORMATION FOR SEQ ID NO: 7

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 248
 (B) TYPE: nucleic acid
 (C) STRANDENDESC: Jack

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TTCGGAGG 248	GCTGGGCCAG GCCTTCCTGG ACTGCTGCTG CTGCTGCTGC TGTNAGGAGT GCGGGGGGGC	GTTCTCCACC TTCTTCAAGG GCGCCATCAC CCCAGTGCTG CTCCTTTGCA TCTGCAGGCC 180	GGCCTACCTC TCCACCGAGC TGACCCGGCA GNCCCTGGAC GTCCTGGGCC TCATCAACCA 120	AACAAGGGCC GIGGICIACG NCIICIGCAC CCICCCANAG AACGICIGCA ACAICGIGGI 60			(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 6:	(11) MULECULAR TYPE: CDNA	(D) TOPOLOGY: linear		(A) LENGTH: 248	(+) SEQUENCE CHARACTERISTICS:
88			20		15			10			U n	
(D) TOPOLOGY: linear		(1) SEQUENCE CHARACTERISTICS:(A) LENGTH: 28	INFORMATION FOR SEQ ID NO: 8		AAGITGGCTG TCATCTGGGT GGGCTC 28	(A1) SEQUENCE DESCRIPTION: SEQ ID NO: 7:		(ii) MOLECULAR TYPE: synthetic DNA		(B) TYPE: nucleic acid	(A) LENGTH: 26	(4) CEOURING ON STORY

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8: (11) MOLECULAR TYPE: synthetic DNA TGAGCICCIG CIGICGCAGC TGCCACAG ENGTH: 28

YPE: nucleic acid

TRANDEDNESS: single

OPOLOGY: linear ION FOR SEQ ID NO: 8 ENCE CHARACTERISTICS: 28

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INFORMATION FOR SEQ ID NO: 9

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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- (ii) MOLECULAR TYPE: synthetic DNA
- (x1) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CATGCGGCG TTCTGGTAGG TCATCAC 27

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INFORMATION FOR SEQ ID NO: 10	INFORMATION FOR SEQ ID NO: 13
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 28 (B) TYPE: nucleic acid (C) STRANDEDNESS: sIngle	(1) SEQUENCE CHARACLEAGES (1) (A) LENGTH: 33 (B) TYPE: nucleic acid (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:	(*1) SEQUENCE DESCRIPTION: SEQ ID NO:
GAAGAGGATG GGCAGGCAGA AGTAGCAG 28	GICGACGAGA IGIGIGAGGG CAGCAAAGAG IGC
INFORMATION FOR SEQ ID NO: 11	
ISTICS: id ngle	(i) SEQUENCE CHARACLERIZED (A) LENGTH: 28 (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
itic DNA	(ii) MOLECULAR TYPE: synthetic DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11: ATGAAGGGCA CGCCACGACA AGAAACG 27	
	35 TUPORWATION FOR SEQ ID NO: 15
INFORMATION FOR SEQ ID NO: 12 (1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 (B) TYPE: nucleic acid (C) STRANDEDNESS: single	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
2 i	(11) MOLECULAR TYPE: Synthetic DNA (xi) SEQUENCE DESCRIPTION: SEQ ID N
-	so GICGACIGGC IGICICCIGG ICAICGAGCC ['] AI

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- ID NO: 13:

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- DNA
- Q ID NO: 14:

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- DNA
- EQ ID NO: 15:

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A G-protein coupled receptor protein which comprises the same or substantially the same amino acid sequence
as that represented by SEQ ID NO: 1, or a variant of the amino acid sequence having a deletion, addition or

substitution of 1 to 30 amino acids, or its salt

The G-protein coupled receptor protein of claim 1 which comprises the same or substantially the same amino acid sequence as that represented by SEQ ID NO: 2, or a variant of the amino acid sequence having a deletion, addition or substitution of 1 to 30 amino acids, or its salt.

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- A partial peptide of the G-protein coupled receptor protein of claim 1 or its salt.
- An isolated DNA comprising DNA having a nucleotide sequence encoding the G-protein coupled receptor protein of claim 1.

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- 5. The isolated DNA of claim 4 having the nucleotide sequence represented by SEQ ID NO: 3.
- The isolated DNA of claim 4 having the nucleotide sequence represented by SEQ ID NO: 4.
- A recombinant vector comprising the DNA of claim 4.

A transformant comprising the recombinant vector of claim 7.

- The transformant of claim 8 which is <u>Escherichia coli</u> HB101/pHEBF2 (FERM BP-5724).
- A process for preparing the G-protein coupled receptor protein of claim 1 or its salt which comprises cultivating the transformant of claim 8 to form the G-protein coupled receptor protein and recovering it.
- 25 11. A method for determining a ligand to the G-protein coupled receptor protein of claim 1 or its salt which comprises bringing the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or its salt into contact with a test compound.
- A method for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein
 of claim 1 or its salt, or their salts which comprises comparing
- (i) ligand binding upon bringing the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or its salt into contact with the ligand, and
- (ii) that upon bringing the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or its salt into contact with the ligand and a test compound.

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13. The screening method according to claim 12, wherein ligand binding is compared by means of an amount of a labeled ligand bound to the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or its salt; an amount of a labeled ligand bound to cells containing the G-coupled receptor protein or a membrane fraction of cells containing the G-protein coupled receptor protein of claim 1; an amount of a labeled ligand bound to the G-protein coupled receptor protein of ealim 1 expressed on a cell membrane of the transformant of claim 8; or cell stimulation activities mediated by the G-protein coupled receptor protein of claim 1.

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- 14. A kit for screening for compounds which alter binding of a ligand to the G-protein coupled receptor protein of claim to rits salt, or their salts which comprises as an essential component the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or its salt.
- 15. The kit of claim 14, wherein the component is in the form of cells containing the G-protein coupled receptor protein of claim 1 or its selt or a cell membrane fraction of cells containing the G-protein coupled receptor protein of claim 1 or its selt.
- 16. A compound which alters ligand binding to the G-protein coupled receptor protein of claim 1 or its salt obtained by the screening method of claim 12 or the kit of claim 14.
- 55 17. An antibody against the G-protein coupled receptor protein of claim 1 or its salt or the partial peptide of claim 3 or its salt.

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18. A method for determining the G-protein coupled receptor protein of claim 1, or its salt or the partial peptide of claim

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3 or its salt in a specimen which comprises bringing the antibody of claim 17 into contact with the G-protein coupled receptor protein of claim 1, the partial peptide of claim 3 or a salt thereof in the specimen.

- 19. A pharmaceutical composition comprising as an effective component the G-protein coupled receptor protein of claim 1, or its salt or the partial peptide of claim 3 or its salt, and a pharmaceutically acceptable carrier or diluent.
- A pharmaceutical composition comprising as an effective component the compound of claim 16 or its salt, and a pharmaceutically acceptable carrier or cliuent.

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12 r	TOTOGOTHGIOGGCTTCCTCTCTTGTATCAACTATGTGGCAAGTGACTGAC	120
₹	AACCTCAGATTIGTGATCTGAGATTAATCAAGGGTTAATTGAGAAACCAGCTGAGTGCT	180
4 T	AGCACCTAGTVAGTGTTCAGTAAGTGACAGTGACGGTTATTGCTGAGTCTTGAATGGAGG	240 1
54 T	AGCTOCCTTNGANTCAGGAGACTTGGGCCCAGTTCCCATTCTGCCCCACCTCCTGTGT	700
5 5 1	CACCCTAGGCAGGCACATTTCCTCCCTAGTTTCAGGGGCCTGAAGCAGATGCCCTCTTA	360
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2 4	GTGAGGGCAGCAAAGAGTGATGTGTGCAGCAGAGGGCCCTGCCCGGCCTGTGGGCCGGA	480
84	OSCTODANOGA OGGCA SOCIANTO CENTRACE OCTODA COCOCO OCTODA OCTODANOGA OCTODANOG	340
561 1	CENACTECETETETETESACCENACTETEGGCCCCCTIGINCTCACTICETETECTIGGCC	900
409	rocrorence converse controverse en recentarion de la recentación d	8g 21
199	GTCATTTTCCTTTGCGCTAMCCAGGTCTCTTGCGCGCTCCCCCTGCACTTGCACTGCAC	ន្តដ
ű a	CACACACCERCACCACCACCACACACACACACACAAAAAAAA	82
15 32 X	OCCANDOCCTICENGENGTHITOTICSCTICANGANGTOCOCOGATACCCCCGGCCCATTCAC Alazysolyvalolinolinysvalpreoludiutrpalaciutyrprokegfrollehis	980
14 15	CTIGCTGCCTICCHGCCHACCAACCCTTGGTGGCCCCTAACCCCGAACAAGAAT Proalaglyleaglaptothtlysptgleavalalathtggtpcaanproaplysäsep	25
22	ocoocoloccololocolocolocolocolocolocolo	112
1981	AGGCTACAGATGCAAGGCGGGTGTATGCGGGGAGGGGGGGG	1020 261
123	atcatectactaccectegrapatroccoregocatroegocalectoroccatcat Ilemeleuleual eleuvelvelvelpaal evalgiyil avalgiyasalensefvelmee	1080
15 23	TOCATICOTOTOCACACTACTACTAAAACOCCTCCAACTCCATCCTTOCOACCTO Cys11eValttyHissertytytytleviyeSeralattpasnSerilelevalldSerlev	1140
121	OCCCTCTOCATTITICTOCTCCTCTTTTTCTCCCTATTGTCATCTTCAACAGATC AleleutrpakepPheleuValleuPhePheCycleuProlioYolIlePheasafGluile	1200
1201	ACCAACAGAGGCTACTGGGTAACTTCTTGTGGGGGGGCGTCCCCTTCATGAAGGCGTUCC The Lyselink tgleulend lyksplaiser cysargalaval probhehet Gluval Ser	1260 212
1261	TCTCTGGGAGTCACCACTTTCACCTTCTGTGCCCTGGGCATTCACGGTTCCACGTGGCC SetLeuGlyValThtThtPheSetLeuGyalaLeuGlyLleAsphughheHiaValAla	222
ដ្ឋ	ACCACACCTGCCCAAGGTGAGGCCATCGAGGGGTGCCAATCCATCC	1380 252

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Fig.

20 4	2041 CCACTCCTGCCCCTGGGCCCCAGTA	Ñ
8 4	1981 PACHAGATCHAGACCAGGTGTCCTCTACTACTACTACACAGAGCGGGGGTGTCCC 481 ApartysLeutysThaGluValSerSerSerIlefyxPheHistysProArgGluSerPro	ㅋ
ğ <u>4</u>	1921 TOCTOCTOCTOTOAGGAGTOCOCCOCTTOCGAGGCTCTOCTOCCAATGGGTCGGAC (3) CysCysCysCysCysGluGluCysGlyGlyAlaSerGluAlaSerAlaAlaAsm8lySerAsp	ä
5 4 5	1861 COMOTOCTOCTOCATTOCACTOCACCOCTOGGCCAGGCCTTCCTGGACTGCTGCTGC 413 ProvalleuteuteutyslleCyskysproleuglyGlaAlaPheleukspCysCysCys	ä
186	1801 ACCCTGGACCTCCTGACCACTCTCTCCACCTTCTTCTACAGGCGCCATCACC 883 ThrLeubspleuleuglyleulleasnGldPheSerThrPhePhelysGlyAlalleThr	8
8 8 8	1741 CTCCCAGAGAACTCTOCAACATOGTGGCCGCACCTCTCCACCGAGCTGACCCGCAG	5
F. C.	681 TOTICHCHOCCHOTICHCHCGCGTGGTGGGCCTGACCGTGGTCTACGCCTTCTGCACC 333 CysGluSerGlnLeubsnSerThrValValOyLeuthrValValIyrhlaPheCysThr	დ "
35	611 TOCCOGTICCROCCCTICCROCIACOLAGICACROCROCIACOLACACACACACACACA III TEPATGVALAXGVALAXGCIAFECPTOCIAAFILYSSERCIUCYSAXGAIASEKTLYSHISGIUGIN	· · · · ·
252 33	561 PACTITICSCICCIACITICIOCOTOCOCATOCICTICACAGICACCOCOGCIGGIGAA. 313 TyrpheGlyCysfyrPheCysLeuProlleleuPhethrValThtCysGlnLeuValThr	ທັ‴
32.	501 AGCTIGCCCGAGTCCCTGTATTCACTGGTGATGACCTACCAGAACGCCCGCATGTGGTGG 281 SerieuProGluSerieuTyrSerieuValmetThrTyrGlnAsnAlaArgHetTrpArp	აგ ∾
250	441 GCACAGGAGCCTGCCCCCACCATGGCCACCTGGACTCATGCATCATGGAACCCTCAGCC 773 AlaGlAGluProAlaProThirHetGlyThrLeuAspSerCysIleMetLysProSerAla	4 ~
27.	381 GCTGTCATCTGGGGGGGCTCCAGOGGGGGGGGGGGGGGGG	ฅผ

601 TOCHTOTTCTRETEATCOACCARDOGOTGCTGTGCCCCTUGCTTGCTTGCT 53 TTPLeuSerProblaidsFroblaidsUkgTtpLeuTtpProbeuAlaValSerLoubla

421 GTO-LOGOCHAMAHATIC/TATOTICHACHAGIAGIACTTICCCCGGCCTGTGGCCGGG

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361 GOSCCCCHCAGCCTGACATCCTGTAGGGCCTTAGAAGGGCGTGGTGGAGGACGAGATGT 301 CACCCTAGGCAGGCACATTTCCTCCCTAGTTTCLGGGGGCTTGAAGGAGGATGCCCTCTTA 241 AGCTGCCTTAGAATCAGGAGACCTGCGCCCCCAGTTCCCATTCTGCCCCCACCTCCCTGTGT 181 AGCACCTAGTAAGTGTTCAGTAAGTGACAGTGATTGCTGAGTCTTGAATTGGAGG

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1121 ACCAGCACCTOCCCAAGATCAGGCCATCGAGGGGTGCCAATCCACCCAGCAAGTTG 193 ThrSetThrLeutroLynValktyBrolleGluktyCysGlnSetIleLeuklalysLeu 1161 TETETOSSAGTEACHACTETOTOCCCTOSSCATTOLCCACTTOCACOTOCCC
271 SerLeuciyye1ThrThrPheSerLeuciysAlaleuciy11aAcpAcgPheHisVallal 1101 ACCAACHCAGCTACTGGTTGAGTTGAGTTCTTGTTGTGGGGGTGGGCTTGATGGAGGTCTGC 253 ThruysGlaArgueuLeuGlyAspVelSesCysArgAlaValProPheleosluvalSes 1141 OCCUPIOGRATHTHENGOTETTETTTATETCETECTTATOTEATETTTAACGAGATE - 213 ALGLEOTTPASPRILEAVALLAUTHERHOSYSLEUPTOLLEVALLIE TAAAGGLULLE 1981 TECHTOTOTOCOLOACTRICHOCTOMOMOCOCCTGAMCTTCCATCCTTGCCACCTTG
213 Cys11eVe17tpHisSerfyrfyricellysSerAleTtpAnSert1eLeuMaSerLeu 1021 AFCATOCTTCTGGGGCTGGTGGTGTTTGCGGTGGGCATTGTGGGCAACCTGTGGGTCATG
193 TleisetlendeuAlatenValValPhaAlaValGlyLlevalGlyAsnLeuSerValDat 961 AGCERASHATUHGAACCCTTTATCCGGTUACCGAGACCTCCTRCAGTGCCTATGCC 173 ACHAGCHTLAGLAAACCCCTTTATCCGTUACCGAGACCTCCTCCAGTGCCTATGCCTATGCC 901 GOGOZOACCCCAGACHGTOGGAGAACTGAGGGGCAATCTGAGAGGGCACCAGGGCAG 153 GlyGlyffirProkspSerGlyGlnGluLeuArgGlyAsmLevffirGlyAlaProGlyGla 841 CCTRETROCCTREMACCUMCUMOCCCTRECTROCCMCCMCCTRACCCCTRACCCCGHCAMGANT 133 ProhladlyLeadInProffirItysProlesNalMalafthtSerProhenProheplysAap 661 oftoartinoochonocochaochosantinosocafochochochonochoochos 73 ValiieleuklaValolyteuserkepValserolyolyalaProteuklatavolyarg

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121 AANOCTCHGATTTGTGATCTGAGATTAATCAAGGGTTAATTGAGAAACCAGCTGAGTGCT

61 TOTOGGTTGTGGGCTTGCTCTGCCTTGTATCAACTATGTGGCAAGTGACCTGTAC

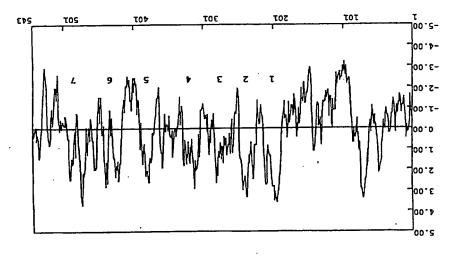
62

Fig. 4

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Fig. 3 (continued)

2080	2041 CCACTCCTGCCCTGGGCACATCTTGCTGAGGCCCAGTA 533 ProLeuleuProLeuGlyThrProCys***	20
2040 533	1981 AACAAGCTCAAGACGAGGTGTCTTCTTCCATTCTACACACAC	ង្គស
1980 513	1921 TGCTGCTGTGTGAGGAGTGCGGCGGGGGCTTGGGAGGCCTCTGCTGCCAATGGGTGCGAC 493 CysCysCysGlyGluCysGlyGlyAlaSerGluAlaSerAlaAlaAsnGlySerAsp	4
1920 493	1861 CCAGTECTECTECTTGCALCTGCAGGCCGCTGGGCCAGGCCTTCCTGGACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGGTAATAPheleukspCysCysCysCysCysCysCysCysCysCysCysCysCysC	28
473	801 ACCCTGGACTCCTGGGCCTCATCAACAGTTCTCCACCTTCTTCAAGGGGGCAATCACC 453 ThrleubspleuleuglyleuileasaglapheSerthrphephelysGlyalailethr	1801
1800	741 CTCCCAGAGAAGGTCTGCAACATCGTGGTGGCCTACCTCTCCACGGAGGTGACCTGGCAG 431 LeulyroGluAsnValCysAsm11eValValAlafyr1euSerfhrGluLeufhrArgGln	1741 433
1740 433	1 totalasaccascttcacacaccotootooccaccotootoataaasaccttctocacc 3 cysõiuserolnieuksnserthirvalvalolykeuthirvalvalvaltyralaeheCysthir	1681 413
1680	1 TOCOCOCTOCAGCCACCAGGAACTCAGAGTCCAGGCAGCAGCAGAGAGAG	1621 393
1620 393	1 TACTTIGGCTGCTACTTCTGCCTGCCCATCTCACAGTCACCTGCCAGTGGTGACA 3 TyrpheGlyCysTyrPheCysLeuProlleLeuPheThrValThrCysGlnLeuValThr	1561 373
1560 373	L ACCTICCCCACTCCTGTATTCACTGOTCATCACCTACCACAAAGGCCCGCATGGGTGG 3 SerLeuptcGluserLeufytserleuvalNetThtTytGlaasaAlaargkelTtyTtp	1501 353
1500 353	GCACAGAGACCTGCCCCACCATGGGCACCCTGGACTCATGCATG	1441 333
333	. GCTGTCATCTGGGTGGGCTCCATGAGGCTGGGTGTGCTGAGCTCGTGCTGTGGCAGTG . AlaVallleTrpValGlySerMetThrLeualaValProGluLeuLeuLeuTrpGlnLeu	1381 313



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4 25.r
                                                   LungLiverSkeletal MuscleKidney
                          · Heart
· Brain
· Placenta
· Pancreas
```

- · Spleen · Thymus · Prostate

- Testis
 Uterus
 Small Intestine
 Colon
 Peripheral Blood
 Leukocyte
- · Gerebellum
 · Cerebral Cortex
 · Medulla
 · Occipital Pole
 · Frontal Lobe
 · Temporal Lobe
 · Putamen
 · Spinal Cord

- · Amygdala
 · Caudate Nucleus
 · Corpus Callosum
 · Hippocampus
 · Whole Brain
 · Substantia Nigra
 · Subhalamic Nucleus
 · Thalamus

